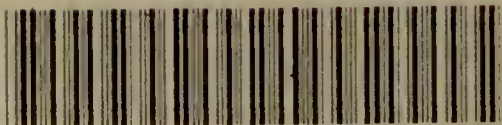


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PTOMAÏNS, LEUCOMAÏNS, TOXINS AND ANTITOXINS:

OR

THE CHEMICAL FACTORS IN THE CAUSATION OF DISEASE.

BY

VICTOR C. VAUGHAN, PH.D., M.D.,

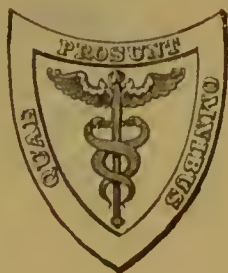
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THIRD EDITION, REVISED AND ENLARGED.



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TO

ALBERT B. PRESCOTT, PH.D., M.D., F.C.S.,
DIRECTOR OF THE CHEMICAL LABORATORY IN THE UNIVERSITY OF MICHIGAN,

THIS LITTLE WORK

IS RESPECTFULLY DEDICATED

AS A SLIGHT TOKEN OF THE HIGH ESTEEM IN WHICH

HE IS HELD BY HIS FORMER STUDENTS,

THE AUTHORS.

PREFACE TO THIRD EDITION.

THE chemistry of disease grows in importance each year. It is now generally recognized that those diseases that cause the greatest mortality, and consequently are of the greatest importance, are in reality cases of poisoning. The poison may be possessed of life, and, therefore, under favorable conditions, capable of indefinite increase in quantity after its introduction into the body, or it may be dead when introduced or generated in the body. The difference between the living pathogenic bacterium and the same organism deprived of life is one of degree rather than one of kind. A very few living bacilli of anthrax injected under the skin of a susceptible animal cause certain symptoms followed by death. A larger number of the same bacilli, after having been deprived of life, by the action of chloroform or some other agent that does not chemically alter the contents of these cells, injected under the skin of a companion animal of the same species induces exactly the same symptoms and causes death in the same manner. The difference is solely one of quantity. Pathogenic germs are living poisons, and every infectious disease is actually an intoxication.

Not only are there chemical factors in the causation of disease, but specific chemical agents are now being employed in the prevention and cure of disease. Inasmuch as both of

these classes are discussed in this volume, the title of the book, "The Chemical Factors in the Causation of Disease," is not altogether appropriate. However, understanding that the scope is somewhat wider than its name would imply, we place this edition before the profession, hoping that it will meet with the same kind reception that has been awarded its two predecessors.

UNIVERSITY OF MICHIGAN, April, 1896.

PREFACE TO SECOND EDITION.

THE preparation of this edition has been made a work of pleasure on account of the many kind words which have been said concerning our first effort to collect the scattered facts pertaining to the chemical factors in the causation of disease. We must be allowed to express our gratification at the general acceptance accorded to the statements which we first made three years ago, and which were then regarded by many as extremely radical. At that time many of the leading bacteriologists held to the "mechanical interference" theory, and regarded the chemical products of germs as of some interest, but in no direct way concerned in the causation of disease. Now the fact that a germ is pathogenic is considered to be sufficient evidence that it elaborates poisonous products, and the study of these products is regarded as of the greatest importance in the investigation of the germ and the disease which it causes. The interest in this subject is not confined to a study of the causation of disease, but efforts are being made to secure immunity from disease and even to effect cures by the employment of the bacterial products. This line of study has certainly become one of great interest to all scientific students of medicine.

In the preparation of the present edition we have endeavored

to utilize the latest and best information, and we can only express our thanks for the encouragement which we have received from so many sources and hope that the present effort will justify no censure.

UNIVERSITY OF MICHIGAN, September, 1891.

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PREFACE TO FIRST EDITION.

WITHIN the past ten years much has been said and written concerning the basic substances formed during the putrefaction of organic matter, and those which are produced by the normal tissue-changes in the living organism. Many investigators have given their whole time and attention to the study of these substances, and important discoveries have been made and much light has been thrown upon what have heretofore been considered problems in medical science. To collect, arrange, and systematize the facts concerning ptomaines and leucomaines have been our first object. Although many short essays, some of them of great value, have been written with the above-mentioned object in view, the present work may be regarded as the first attempt to make this collation embrace everything of importance on this subject. In endeavoring to accomplish this object we have met with many difficulties. The original reports of the various investigators are scattered through the pages of medical and scientific journals, transactions of societies, monographs, government reports, etc. However, with few exceptions we have been able to obtain the original reports, and we think that we have included everything of importance published up to the present year (1888).

To the physician the facts which have been made known concerning the putrefactive and physiological alkaloids must

be of great value, and if this little work furnishes the means by which members of the profession may become better acquainted with the nature of those poisons which are introduced from without, and those which are generated within the body of man, the object of its authors will be accomplished.

UNIVERSITY OF MICHIGAN, July, 1888.

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PTOMAINS, LEUCOMAINS, TOXINS, AND ANTITOXINS.

INTRODUCTION.

THE CAUSATION AND CLASSIFICATION OF DISEASES.

THE animal body is made up of groups of specialized cells, which are mutually dependent one upon the other for their normal development and continued healthy existence. These groups of cells constitute the various organs and the framework by which they are held together and through which food is distributed to all, and the special products of one colony are carried to the others or cast out from the whole as waste material. Each organ has its special functions, the healthy performance of which is necessary to the well being of the whole. If the digestive cells of the alimentary canal fail to secrete active fluids, the cells of the brain, heart, lungs, and, in short, of every part of the body, are impaired, and disease results. If the food be properly digested and the absorption cells of the walls of the alimentary canal fail, a like disaster is brought upon the whole. If the kidneys, liver, skin, or lungs fail to eliminate effete and poisonous products, these accumulate and interrupt the healthy activity of the cells of the body. When the pancreas is extirpated, or when it has atrophied after its duct has been closed, or when it has become structurally diseased, the formation of glycogen in the liver and elsewhere is interrupted and diabetes results.

Disease is the result of impaired or perverted cell action. The causes of disease are to be found in those agencies which induce this impaired or perverted cell action.

The body is often invaded by foreign cells, which may become, for a time at least, parasites, living at the expense of the host and elaborating their own special products, which are harmful to one or more of the colonies of cells constituting the healthy body, and thus place in jeopardy the health and even the existence of the whole. These cells find their way into the body with the food, drink, or inhaled air, or through some break in the skin or mucous membrane. These harmful invaders may consist of microscopic, unicellular forms of vegetable life known as bacteria, and the action of their special products upon the cells of the body gives rise to the *bacterial* diseases.

Certain other vegetable forms of life, especially those belonging to the fungi, live as parasites on the higher animals. These are not known to produce chemical poisons, but by their presence and encroachment on certain tissues they induce impaired or perverted action of the cells of the same. The skin is the favorite habitat of these parasites, and the disorders which they cause are known as *fungous* diseases.

Some species of single-celled animal organisms, known as protozoa, invade the body and there live and reproduce themselves, modifying, impairing, and destroying normal tissue. The disorders resulting from these causes are known as *protozoal* diseases.

Some more highly developed animals pass, at least, a portion of their lives as parasites, and we must recognize certain diseases as due to *animal parasites*.

The living cells of the animal body may be altered or destroyed by the action of poisons of mineral, vegetable, or animal origin. This poisoning may be acute or chronic; it may manifest itself in one case principally by its action on the nervous system, and in another the symptoms induced may be referred more especially to the digestive organs. Diseases due to the administration of poisons generated wholly

outside the body are grouped together under the name of *intoxications*.

A given group of cells may be so altered by mechanical violence that the continued performance of healthy function is no longer possible. A depression of the skull, as the result of a fall or blow, may induce epilepsy or insanity. Diseases induced in this manner are said to be *traumatic*.

Lastly, without outside interference, any group of cells in the body may, from having an excess of work thrown upon it, or from other causes, many of which remain unknown, fail to do its duty, and, as a consequence, disaster may threaten the whole. These diseases may be denominated as *autogenous*.

This gives us a simple etiological classification of diseases into: 1. Bacterial. 2. Fungous. 3. Protozoal. 4. Animal parasitic. 5. Intoxications. 6. Traumatic. 7. Autogenous.

CHAPTER I.

DEFINITION AND CLASSIFICATION OF THE BACTERIAL POISONS.

PTOMAINS.—An exact classification of the chemical factors in the causation of the infectious diseases can probably not be made at present. We know of two chemically distinct classes, one of which contains substances which combine with acids, forming chemical salts, and which in this respect at least correspond with the inorganic and vegetable bases. The members of this class are designated as ptomaines, a name suggested by the Italian toxicologist, SELMI, and derived from the Greek word *πτωμα*, meaning a cadaver. A ptomain may be defined as an organic chemical compound, basic in character, and formed by the action of bacteria on nitrogenous matter. On account of their basic properties, in which they resemble the vegetable alkaloids, ptomaines may be called putrefactive alkaloids. They have also been called animal alkaloids, but this is a misnomer, because, in the first place, some of them are formed in the putrefaction of vegetable matter; and, in the second place, the term “animal alkaloid” is more properly restricted to the leucomaines—those basic substances which result from tissue metabolism in the body. KOBERT employs the term ptomatin as etymologically preferable to ptomain.

While some of the ptomaines are highly poisonous, this is not an essential property, and others are wholly inert. Indeed, the greater number of those which have been isolated up to the present time do not, when employed in single doses, produce any apparently harmful effects. BRIEGER restricts the term ptomain to the non-poisonous basic products, and designates the poisonous ones as “toxins.” This is a classi-

fication, however, which seems to be of questionable utility. It is not always easy to say just what bodies are poisonous and what are not. The poisonous action of a substance depends upon the condition under which, and the time during which, it is administered. Thirty grains of quinin may be taken by a healthy man during twenty-four hours without any appreciably ill effect, yet few of us would be willing to admit that the administration of this amount daily for three months would be wise or altogether free from injury. In the same manner the administration of a given quantity of a putrefactive alkaloid to a dog or guinea-pig in a single dose may do no harm, while the daily production of the same substance in the intestine of a man and its absorption continued through weeks and possibly months may be of marked detriment to the health. We do not as yet know enough about the physiologic or toxicologic action of the putrefactive alkaloids to render the classification proposed by BRIEGER worthy of general adoption. Besides, this term "toxin" is now quite generally, although somewhat incorrectly, employed to designate those non-basic bacterial poisons for which BRIEGER suggested the name "toxalbumin."

All ptomaïns contain nitrogen as an essential part of their basic character. In this they resemble the vegetable alkaloids. Some of them contain oxygen, while others do not. The latter correspond to the volatile vegetable alkaloids, nicotin and coniin, and the former correspond to the fixed alkaloids.

Since all putrefaction is due to the action of bacteria, it follows that all ptomaïns result from the growth of these microorganisms. The kind of ptomaïn formed will depend upon the individual bacterium engaged in its production, the nature of the material being acted upon, and the conditions under which the putrefaction goes on, such as the temperature, the amount of oxygen present, and the duration of the process.

BRIEGER found that, although the Eberth bacillus grew well in solutions of pepton, it did not produce any ptomaïns ;

while from cultures of the same bacillus in beef-tea he obtained a poisonous alkaloid. FITZ found that whilst the bacillus butyricus produces by its action on carbohydrates butyric acid, in glycerin it produces propylic alcohol, and MORIN has found amyl alcohol among the products of this germ. BROWN has shown that while the mycoderma aeti converts ethylic alcohol into acetic acid, it converts propylic alcohol into propionic acid, and is without effect upon methylic alcohol, primary isobutylic alcohol, and amylic alcohol. Some bacteria will not multiply below a given temperature. Thus, the bacillus butyricus will not grow at a temperature below 24° .¹ The lower temperature does not destroy the organism, but it lies dormant until the conditions are more favorable for its growth. PASTEUR divided bacteria into two classes—the aërobie and the anaërobie. As the name implies, the former grow and thrive in the presence of air, while the latter find their conditions of life improved by the exclusion of air. Therefore, different ptomaines will be formed in decomposing matter freely exposed to the air, and in that which is buried beneath the soil or from which the air is largely excluded. Ignorance of this fact has led to some serious mistakes in toxicologic work, as we shall see further on. Even when the same ferment is present the products of the putrefaction will vary, within certain limits, according to the extent to which the putrefying material is supplied with air. The kind of ptomain found in a given putrid substance will depend also upon the stage of the putrefaction. Ptomaines are transition products in the process of putrefaction. They are temporary forms through which matter passes while it is being transformed, by the activity of bacterial life, from the organic to the inorganic state. Complex organic substances, as muscle and brain, are broken up into less complex molecules, and so the process of chemie division goes on until the simple and well-known final products, carbonic acid gas, ammonia, and water, result; but the variety of combinations

¹ All temperatures given in this work are centigrade, unless otherwise specified.

into which an individual atom of carbon may enter during this long series of changes is almost unlimited, and with each change in combination there is more or less change in nature. In one combination the atom of carbon may exist as a constituent of a highly poisonous substance, while the next combination into which it enters may be wholly inert.

It was formerly supposed that putrefaction was simply oxidation, but the researches of PASTEUR and others have demonstrated the fact that countless myriads of minute organisms are engaged constantly in transforming matter from the organic to the inorganic form. Lock up the bit of flesh so that these little workers cannot reach it, and it will remain unchanged indefinitely.

It may be asked if any of the changes occurring during putrefaction are to be regarded as purely chemie. Without doubt, many of the secondary products of putrefaction arise from reactions between antecedent and more complex products or by the action of oxygen, water, and reducing agents upon primary products. Ptomains formed in this way may be regarded as the indirect results of bacterial life.

BACTERIAL PROTEIDS.—These substances have been known for so short a time and are at present so imperfectly known that many difficulties arise in discussing them. In the first place, we may divide the bacterial proteids into two classes: 1. Those which constitute an integral part of the bacterial cells; and 2. Those which have not been assimilated by the cells, but which have been formed by the fermentative or cleavage action of the bacteria on the proteid bodies in which they are growing. Even this classification is of questionable value. We allow bacteria to grow for a number of days in a nutrient solution. We then separate the soluble constituents from the formed cells by filtration through porous tile; we wash the latter and then study their proteid contents; but a considerable proportion of the contents of the living cells has already passed into solution, and the bacterial detritus left on the filter gives no exact knowledge of the constituents of the

living cells. Besides, the living cells absorb and excrete, and we are not yet always able to distinguish between those substances formed within the cell and those pre-existing in the culture medium. The filtrate contains, or may contain, any one or more of the following proteid bodies: 1. Those portions of the proteid substances which were used in the preparation of the untrient solution and which have escaped the action of the bacteria; 2. Proteids which have been at one time integral parts of the cells, but which have passed into solution on the death and dissolution of the bacteria; and 3. Proteids which have been formed by the fermentative action of the bacteria, or those which are defined as constituting the second class, as given above. We know at present of no means by which one of these proteids can with certainty be isolated from the others. However, the above classification is a convenient one, and with a clear understanding that it is not free from criticism we may employ it until a more thorough and scientific study of these bodies has been made.

There is no positive proof up to the present writing (1895) that any of the proteids formed by the cleavage action of bacteria on the normal proteids of culture media, or on those of the animal body, are specifically factors in the production of disease. Many bacteria peptonize proteids, but there is no evidence that the peptons thus formed are any more poisonous than those formed by the gastric juice. However, the work that has been done on this subject can hardly be regarded as conclusive. As we shall see later, bacteria form poisons by synthetic rather than by analytic processes. The poisons are formed within the bacterial cells. The constituents of the nutritive media are incorporated in the bacteria before they become specific poisons.

THE BACTERIAL CELLULAR PROTEIDS.—NENCKI first prepared one of these substances from putrefactive bacteria. These were obtained by decantation, freed from fat with ether, dissolved in fifty parts of a potash solution of 0.5 per cent., heated for some hours at 100°, and filtered. The filtrate was

acidified with dilute hydrochloric acid and precipitated by the addition of rock salt. The precipitate was washed with a saturated salt solution, dried at 100° , and washed free from salt with water. NENCKI designates this substance as "mycoprotein," and finds that it has the formula, $C_{25}H_{42}N_6O_9$. Freshly precipitated mycoprotein forms amorphous flakes, which are soluble in water, alkalis, and acids. The aqueous solution is acid in reaction. After being dried at 100° it is no longer wholly soluble in water. NENCKI found that it is not precipitated from aqueous solution by alcohol, but by picric acid, tannic acid, and mercuric chlorid; that it does not give the xanthoproteic, but does give the Millon and the biuret reactions. According to SCHÄFFER, it is changed by acids into pepton, and on being fused with five parts of potash it breaks up into ammonia, amylamin, phenol (0.15 per cent. of its weight), valeric acid (38 per cent.), leucin, and traces of indol and skatol. A proteid obtained from the yeast plant has the formula, $C_{12}H_{21}N_3O_3$.

The purified pyogenetic agent obtained from the pneumonia bacillus of FRIEDLÄNDER was found by BUCHNER to give the following reactions: It is soluble in water and the concentrated mineral acids, very soluble in dilute alkalis, from which it is precipitated on the addition of an acid. From its aqueous solution, it is not precipitated by heat, nor by saturation with sodium chlorid, but is precipitated by magnesium sulphate, copper sulphate, platinum chlorid, gold chlorid, lead salts, picric acid, tannic acid, and absolute alcohol. It gives the xanthoproteic, Millon, and biuret reactions.

In old bouillon cultures the dead disintegrated bodies of the bacteria form a sediment. It is not at all probable that an analysis of this sediment represents fairly the constituents of the living germs. During the disintegration certain constituents of the cell pass into solution, and these soluble substances are probably the most important constituents of the bacterial cells, so far as they are concerned in the causation of disease. Similar processes undoubtedly occur in the body of an animal infected with a pathogenic organism, and here

it must be that the soluble substances cause the symptoms of the disease and death. Indeed, we know this is true, because the "toxins" are soluble bodies.

TOXINS.—When it became known that some of the specific pathogenic germs elaborate both in artificial cultures and in susceptible animals poisonous basic substances, or ptomaines, it was surmised that the symptoms of the diseases induced by these microorganisms were due in all cases to such basic poisons, and chemists labored diligently to isolate from cultures of each germ its special basic product. These labors soon led to the recognition of the fact that the above-mentioned surmise had been hastily drawn. It was found to be true that the symptoms of each and every infectious disease investigated are due to the chemie products elaborated by the activity of the germ, but these chemie products are not, in the majority of these diseases, basic in character, and consequently they cannot be classed among the ptomaines. Indeed, we do not know of any specific infectious disease, the symptoms of which are due solely to basic poisons. In the etiological study of the infectious diseases the ptomaines are of secondary importance among the bacterial poisons. BRIEGER succeeded in isolating from pure cultures of the tetanus bacillus as many as four ptomaines, but the poisonous effects induced by these substances are not comparable in violence with those which follow injections of tetanus cultures from which the bacillus had been removed by filtration. The fact that the filtered culture is more poisonous than any or all of its basic contents necessitates the conclusion that the culture contains some more active constituent.

LOEFFLER, ROUX, YERSIN, BRIEGER and FRAENKEL failed to find any active ptomain in sterilized cultures of the diphtheria bacillus, notwithstanding the fact that these solutions possessed remarkably poisonous properties. Results similar to these have been obtained with the germs of other diseases. What, then, is the nature of the powerful poisons that are formed in cultures of the bacteria of tetanus, diph-

theria, tuberculosis, typhoid fever, anthrax, and other infectious diseases, and to which the symptoms and death are due? In their studies of the diphtheria poison, ROUX and YERSIN thought that it might be a ferment. BRIEGER and FRAENKEL combated this idea, and advanced the belief that the diphtheria poison is an albuminous body. Indeed, they made an ultimate analysis of the precipitated poison, and upon the results of this, together with the occurrence of certain reactions, they based the validity of their conclusions. They proposed that these non-basic bacterial poisons should be called "toxalbumins," and unfortunately this term has been extensively employed. They also believed that these "toxalbumins" originate in the splitting up of the proteid bodies in the culture media by the action of the bacteria. Now, it has turned out that the two facts that seem to be most positively proven in regard to the toxins are: 1. They are not albumins; and 2. They are not formed by the splitting up of the proteids of the culture media.

It is not our intention to discuss the toxins in detail at this time. They will be described under the several infectious diseases, the chemie poisons of which will be spoken of in a subsequent chapter. In 1891, FRIER made an ultimate analysis of a toxin obtained by Vaughan from a toxicogenic germ found in drinking-water. The results obtained by this analysis are as follows: Carbon, 48.46 per cent.; hydrogen, 7.69 per cent.; nitrogen, 13.44 per cent.; phosphorus, 0.69 per cent. Sulphur was absent. The absence of sulphur and the very small per cent. of phosphorus indicate that this toxin was nearly pure.

In 1893, BRIEGER and COHN found that the tetanus toxin, in the purest form in which they could obtain it, contains no phosphorus, and only unweighable traces of sulphur. The trace of sulphur was probably due to the ammonium sulphate used in precipitation.

Later (1895), BRIEGER made an ultimate analysis of the tetanus toxin, so far purified that 0.00000005 gram kills mice, with the following results: Carbon, 52.08 per cent.;

hydrogen, 8.1 per cent.; nitrogen, 15.71 per cent. This purified toxin is not precipitated by ammonium sulphate, and it gives the biuret reaction so imperfectly that BRIEGER feels justified in saying that the coloration is not due to the toxin, but to some proteid impurity.

The above-mentioned facts convince us that the specific bacterial poisons, now generally known as "toxins," are not proteid bodies. As everyone knows, the word toxin simply means a poison, and all poisons are toxins. Poisonous ptomaïns are also bacterial toxins, but we shall employ the term throughout the book to designate the powerful, specific bacterial poisons, the chemie nature of which remains unknown to us. It is better to employ some general term of this kind than to adopt a name that would give the reader an erroneous impression.

USCHINSKY has made a most important contribution to our knowledge of the toxins, inasmuch as he has demonstrated that these substances are not split products formed by the action of bacteria on proteids pre-existing in the culture media, but are synthetic products, and are formed when the germs are grown on culture media containing no proteids. He has grown a number of pathogenic microorganisms, including those of cholera, diphtheria, tetanus, and typhoid fever, in the following menstruum:

Water	1000 parts.
Glycerin	30-10 "
Sodium chlorid	5-7 "
Calcium chlorid	0.1 part.
Magnesium sulphate	0.2-0.4 "
Di-potassium phosphate	2-2.5 parts.
Ammonium lactate	6-7 "
Sodium asparaginate	3.1 "

From these cultures he obtained toxins that were not less virulent than those formed in bouillon-pepton. The tetanus bacillus grows more luxuriantly in this solution when from 1 to 2 per cent. of grape sugar is added, although some have denied that this germ will grow in this medium. However, this is of no importance to us at this time. If the cholera,

diphtheria, and typhoid bacilli form their poisons by synthetic processes it is altogether likely that the tetanus bacillus acts in the same way, even if it should require more complex articles of food.

USCHINSKY did not attempt to isolate the toxins from the proteid bodies which were present in these cultures, and which also must have been formed by the synthetic action of the bacteria. For this reason, the reactions that he obtained from these cultures must not be considered as identical with those of isolated toxins, a distinction which evidently some other investigators have failed to perceive.

FRAENKEL has prepared active tuberculin and mallein from cultures of the respective bacilli grown in media free from proteids. The independent investigations of KÜHNE, PROSKAUER, and WESBROOK give like results.

TICHOMIROFF has shown that nucleinic acid precipitates the toxins of tetanus and diphtheria, but fails to precipitate those of cholera and of streptococcus infection.

CHAPTER II.

HISTORICAL SKETCH OF THE BACTERIAL POISONS.

It must have been known to primitive man that the eating of putrid flesh was liable to affect the health more or less seriously; and when he began his endeavors to preserve his food for further use, instances of poisoning from putrefaction must have multiplied. However, the distinguished physiologist, ALBERT VON HALLER, seems to have been the first to make any scientific experiments concerning the effects of putrid matter upon animals. He injected aqueous extracts of putrid material into the veins and found that death resulted. Later in the eighteenth century MORAND gave an account of the symptoms induced by eating poisonous meat. In the early part of the present century (1808 to 1814) GASPARD carried on similar experiments. His studies were made with the putrid flesh of both carnivorous and herbivorous animals. With these he induced marked nervous disturbances, as stiffness of the limbs, opisthotonos, and tetanus. GASPARD concluded from the symptoms that the poisonous effects were not due to carbonic acid gas or hydrogen sulphid, but thought it possible that ammonia might have part in their production. It is easily seen now that the effects observed by HALLER and GASPARD may have been due to infection in part or altogether. When they injected putrid material they introduced the bacteria, about which they knew nothing, as well as the bacterial products. In 1820 KERNER published his first essay on poisonous sausage, which was followed by a second in 1822. At first he thought that the poisonous properties were due to a fatty acid, similar to the sebacic acid of Thenard, and which originated during putrefaction. Later he modified these views, and believed the poison to be a compound con-

sisting of the sebacie acid and a volatile principle. This may be regarded as the first suggestion as to the probability of the development of a poisonous substance with basic properties in decomposing matter. In 1822, DUPRÉ observed a peculiar disease among the soldiers under his care, who, during the warm and dry summer of that year, were compelled to drink very foul water. The disease thus induced must have been due to infection rather than to intoxication. Later, MAGENDIE, induced by the investigations of GASPARD and the observations of DUPRÉ, made many experiments, in which dogs and other animals were confined over vessels containing putrid animal matter and compelled constantly to breathe the emanations therefrom. The effects varied markedly with the species of animal and the nature of the putrid material, but in some instances symptoms were induced which resembled closely those of typhoid fever in man. LEURET directed his attention to the chemie changes produced in blood by putrefaction, but accomplished nothing of special value. DUPUY injected putrid material into the jugular vein of a horse, and with TROUSSEAU studied alterations produced in the blood by these injections.

During the third decade of the present century there were many investigators, in addition to those mentioned above, who endeavored to ascertain the active agent in poisonous foods. DANN, WEISS, BUCHNER, SCHUMANN, CADET DE GASSICOURT, and ORFILA studied poisonous sausage, but made no advance upon the work done by KERNER. HENNEMAN, HÜNNEFELD, WESTRUMB, and SERTÜRNER made contributions concerning poisonous cheese, but all believed the caseic acid of KERNER to be the poisonous principle.

In 1850, SCHMIDT, of Dorpat, made some investigations on the decomposition products and volatile substances found in cholera stools; and, two years later, MEYER, of Berlin, injected the blood and stools of cholera patients into lower animals. In 1853, SICH made an important contribution on the effects of acute poisoning with putrid material. He ascertained that, when given in sufficient quantity, putrid matter

produces an intestinal catarrh, with choleraic stools. Nervous symptoms, trembling, unsteady gait, and, finally, convulsions were also observed. STRICH made careful post-mortem examinations, and was unable to find any characteristic or important lesions. Theoretically, he concluded that the putrid material contained a ferment which produced rapid decomposition of the blood.

In 1856 PAXTON published a most important contribution to the knowledge of the nature of the poison present in putrid flesh. He first demonstrated positively the chemie character of the poison, inasmuch as he showed that the aqueous extract of the putrid material retained its poisonous properties after treatment which would insure the destruction of all organisms. His conclusions were as follows :

1. "The putrid poison contained in the decomposed flesh of the dog, and which is obtained by extraction with distilled water and repeated filtration, is not volatile, but fixed. It does not pass over on distillation, but remains in the retort.

2. "The putrid poison is not destroyed by boiling, nor by evaporation. It preserves its poisonous properties even after the boiling has been continued for eleven hours, and after the evaporation has been carried to complete desiccation at 100°.

3. "The putrid poison is insoluble in absolute alcohol, but is soluble in water, and is contained in the aqueous extract which is formed by treating with distilled water the putrid material which has previously been dried by heat and washed with alcohol.

4. "The albuminoid substances which frequently are found in putrid fluids are not in themselves poisonous only so far as they contain the putrid poison fixed and condensed upon their surfaces, from which it can be removed by repeated and careful washing.

5. "The intensity of the putrid poison is comparable to that of the venom of serpents, of curare, and of certain vegetable alkaloids, inasmuch as 0.012 of a gram of the poison, obtained by extracting with distilled water putrid material which had been previously boiled for a long time, dried at

100°, and submitted to the action of absolute alcohol, was sufficient to almost kill a small dog."

PANUM made intravenous injections with this poison, and with ammonium carbonate, ammonium butyrate, ammonium valerianate, tyrosin, and leucin, and found that the symptoms induced by the putrid poison differed from those caused by the other agents. Moreover, he found the symptoms to differ from those of typhoid fever, cholera, pyæmia, anthrax, and sausage poisoning. He was also in doubt as to whether the poison acted directly upon the nervous system, or whether it acted as a ferment upon the blood, causing decomposition, the products of which affected the nerve-centres; but he was sure that it could not correspond to the ordinary ferments, inasmuch as it was not decomposed by prolonged boiling nor by treatment with absolute alcohol. Certainly, the putrid poison could not consist of a living organism.

The symptoms observed by PANUM varied greatly with the quantity of the poison used and the strength of the animal. After the intravenous injection of large doses, death followed in a very short time. In these cases there were violent cramps, and involuntary evacuations of the urine and feces; the respirations were labored, the pallor was marked, sometimes followed by cyanosis, the pulse feeble, the pupils widely dilated, and the eyes projecting. In these cases the autopsy did not reveal any lesion, save that the blood was dark, imperfectly coagulated, and slightly infiltrated through the tissue. Postmortem putrefaction came on with extraordinary rapidity.

When smaller doses or more vigorous animals were used, the symptoms did not appear before from a quarter of an hour to two hours, and sometimes even later. In these cases the symptoms were less violent, and the animal generally recovered. In all instances, however, the disturbances were more or less marked.

In addition to the "putrid poison," PANUM obtained a narcotic substance, the two being separated by the solubility of the narcotic in alcohol. The alcoholic extract was evap-

orated to dryness, the residue dissolved in water and injected into the jugular vein of a dog. The animal fell into a deep sleep, which remained unbroken for twenty-four hours, when it awoke apparently in perfect health. This is of special interest on account of the later researches of BOUCHARD, who has shown that normal urine contains a narcotic substance that can be extracted from the residue obtained by evaporation with alcohol.

PANUM's first contributions, which were published in Danish, did not attract the attention which they deserved, until after the lapse of several years. Now, however, their importance is fully appreciated, and the distinguished investigator lived to receive the credit and honor due him.

WEBER, in 1864, and HEMMER and SCHWENNINGER, in 1866, confirmed the results obtained by PANUM; and SCHWENNINGER announced that in the various stages of putrefaction different products are formed, and that these vary in their effects upon animals. In 1866, BENGE JONES and DUPRÉ obtained from the liver a substance which in solutions of dilute sulphuric acid gives the blue fluorescence observed in similar solutions of quinin. To this substance they gave the name "animal chinoidin." Subsequently, the same investigators found this substance in all organs and tissues of the body, but most abundantly in the nerves. Its feebly acid solutions give precipitates with iodine, potassio-mercuric iodide, phospho-molybdic acid, gold chloride, and platinum chloride. From three pounds of sheep's liver, they obtained three grams of a solution in which, after slight acidulation with sulphuric acid, the intensity of the fluorescence was about the same as that of a similarly acidulated solution of quinin sulphate which contained 0.2 gram of quinin per litre. Still later, this base was obtained by MARINO-ZUCO. It is probably the product of fluorescing bacteria.

In 1868, BERGMANN and SCHMIEDEBERG separated, first from putrid yeast, and subsequently from decomposed blood, in the form of a sulphate, a poisonous substance which they named sepsin. The sulphate of sepsin forms in needle-shaped

crystals. Small doses (0.01 gram) of this substance were dissolved in water and injected into the veins of two dogs. In a short time it produced vomiting, and later diarrhœa, which, in one of the animals, after a time, became bloody. Postmortem examination showed in the stomach and intestines, bloody ecchymoses. It was now believed that the "putrid poison" of PANUM had been isolated, and that it was identical with sepsin, but further investigations showed that this was not true. There are marked differences in their effects upon animals, and sepsin has not been found to be generally present in putrid material. It is only rarely found in blood, and the closest search has failed to show its presence in pus. BERGMANN, following the same method he had used in extracting this poison from yeast, has been unable to obtain it from other putrid material. Moreover, he was not always successful in obtaining the poison from yeast. Sepsin was not obtained in quantity sufficient to serve for an ultimate analysis, hence, its composition remains unknown.

Recently LEVY, working under Schmiedeberg's directions, has made an additional study of putrid yeast, in which he found bacilli resembling those of mouse septicæmia and the proteus vulgaris. Dogs were treated with intravenous injections of liquefied gelatin cultures of the proteus, and the symptoms of sepsin poisoning followed. Mice and rabbits were found to be yet more susceptible to the poison. The cultures were precipitated with absolute alcohol, and the resulting albuminous precipitate caused the same symptoms as the cultures in mice, rabbits, and dogs.

The same author had the opportunity of investigating cases of meat-poisoning due to infection with the proteus. The keeper of a restaurant and some of his guests suffered from a most violent purging, which in the case of the host terminated fatally. In the vomited matter, in the stools, and in the bottom of the ice-box in which the meat was kept, the proteus was found, and cultures of it produced the symptoms of sepsin poisoning in animals. LEVY concludes that the proteus is the generator of sepsin. If this be true the poisonous effects

observed by BERGMANN and SCHMEDEBERG were not due to the crystals obtained by them, but to albuminous bodies. In other words, the sepsin of LEVY is not a ptomain.

In 1869 ZÜLZER and SONNENSCHNIG prepared from decomposed meat extracts a nitrogenous base, which in its chemie reactions and physiologic effects resembled atropin and hyoseyamin. When injected under the skin of animals it produced dilatation of the pupils, paralysis of the muscles of the intestines, and acceleration of the heart-beat; but it was uncertain and inconstant in its action. A similar substance has also been obtained from the bodies of those who have died from typhoid fever, and it may be possible that the belladonna-like delirium which frequently characterizes the later stages of this disease is due to the ante-mortem generation of this poison within the body.

Since 1870 many chemists have been engaged in making investigations on the products of putrefaction. We can only mention a few names at present, while others will be referred to subsequently in discussing the individual ptomains.

First of all stands the Italian SELMI, who suggested the name ptomain, and whose researches furnished us with much information of value, and, what is probably of more importance, gave an impetus to the study of the chemistry of putrefaction, which has already been productive of much good and gives promise of much more in the future. SELMI showed that ptomains could be obtained (1) by extracting acidified solutions of putrid material with ether; (2) by extracting alkaline solutions with ether; (3) by extracting alkaline solutions with chloroform; (4) by extracting with amylie alcohol; and (5) that there yet remained in the solutions of putrid matter ptomains which were not extracted by any of the above-mentioned reagents. In this way he gave some idea of the great number of alkaloidal bodies which might be formed among the products of putrefaction, and the promising field thus discovered and outlined was soon occupied by a busy host of chemists. In the second place, he demonstrated the fact that many of the ptomains give reactions

similar to those given by the vegetable alkaloids. This led the toxicologist into investigations, the results of some of which we will ascertain further on.

SELMÉ, however, did not succeed in isolating completely a single putrefactive alkaloid. All his work was done with extracts. He remained ignorant, except in a general way, of the composition of these bodies. NENCKI, in 1876, made the first ultimate analysis and determined the first formula of a ptomain. This was an isomer of collidin, which will be described later.

RÖRSCH and FASSBENDER, in a case of suspected poisoning, obtained by the Stas-Otto method a liquid which could be extracted from acid as well as alkaline solutions by ether, and which gave all the general alkaloidal reactions. They were unable to crystallize either extract by taking it up with alcohol and evaporating. The colorless aqueous solution was not at all bitter to the taste. The precipitate formed with phospho-molybdic acid dissolved on the application of heat, giving a green solution, which became blue on the addition of ammonia. They believed that this substance was derived from the liver, since fresh ox-liver, treated in the same manner, gave them an alkaloid which could be extracted with ether from acid as well as from alkaline solutions. GUNNING found this same alkaloid in liver-sausage from which poisoning had occurred. RÖRSCH and FASSBENDER state that while in some of its reactions this substance resembles digitalin, it is distinguished from this vegetable poison by the failure of the ptomain to give the characteristic bitter taste.

SCHWANERT, whilst examining the decomposing intestines, liver, and spleen of a child that had died suddenly, perceived a peculiar odor and obtained by the Stas-Otto method (ether extract from an alkaline solution) small quantities of a base, which was distinguished from nicotine and coniine by its greater volatility and its peculiar odor. He supposed that this substance was produced by decomposition, and, in order to ascertain the truth of his supposition, he took the organs of a cadaver that had lain for sixteen days at a temperature of

30° and was well decomposed. These were treated with tartaric acid and alcohol. The acid solution was first extracted with ether, and yielded no result; it was then rendered alkaline and extracted with ether. The latter extract gave, on evaporation, the same substance which he had found in the organs of the child. The residue was a yellowish oil, having an odor somewhat similar to propylamin. It was repulsive, but not bitter to the taste, and alkaline in reaction. On the addition of hydrochloric acid it crystallized in white needles, which were freely soluble in water, but soluble with difficulty in alcohol. On the addition of ammonium hydrate to this crystalline substance a white vapor of unpleasant odor was given off. The crystals dissolved in sulphuric acid, forming a solution which was at first colorless, but which gradually became dirty brownish-yellow, and grayish-brown on the application of heat. On being warmed with sodium molybdate a splendid blue color, becoming gradually gray, was produced. Potassium bichromate and sulphuric acid gave a reddish-brown, then a grass-green color. Nitric acid gave a yellow color. A tartaric acid solution of the crystals produced, on the addition of platinum chlorid, a dirty-yellow precipitate of small six-sided stars, which contained 31.55 per cent. of platinum. Gold chlorid gave a pale yellow, amorphous precipitate; mercuric chlorid yielded white crystals; potassio-mercuric iodid a dirty-white precipitate; and potassio-cadmic iodid yielded no result. Tannic acid produced only a turbidity. Sodium phospho-molybdate gave a yellow, flocculent precipitate, which became blue on the addition of ammonium hydrate. This base has a slight reducing power, and in this it resembles a substance obtained by SELMI, but it differs from SELMI's extract inasmuch as it does not give a violet coloration on being warmed with sulphuric acid. In its amorphous character, its behavior to the general alkaloidal reagents, and its lack of bitter taste, it resembles the base obtained by RÖRSCH and FASSBENDER, but, unlike that alkaloid, it is extractable from alkaline solutions only.

SELM, in commenting upon the base studied by RÖRSCH and FASSBENDER, SCHWANERT, and himself, believing that all were dealing with the same body, states that it does not contain phosphorus, and that it is separated with extreme difficulty from the vegetable alkaloids.

LIEBERMANN, in examining the somewhat decomposed stomach and intestines in a case of suspected poisoning, found an alkaloidal body which was unlike that studied by the chemists mentioned above, inasmuch as it was not volatile. The Stas-Otto method was employed. The ether extract from alkaline solution left, on evaporation, a brownish, resinous mass, which dissolved in water to a turbid solution, the cloudiness increasing on heating. This reaction agrees with coniin, but the odor differed from that of the vegetable alkaloid. The aqueous, strongly alkaline solution gave the following reactions:

1. With tannic acid, a white precipitate.
2. With potassium iodid, a yellowish-brown, turning to dark-brown precipitate.
3. With chlorin water, a marked white cloudiness.
4. With phospho-molybdic acid, a yellow precipitate.
5. With potassio-mercuric iodid, a white precipitate.
6. With mercuric chlorid, a white cloudiness.
7. With concentrated sulphuric acid, after a while, a reddish-violet coloration.
8. With concentrated nitric acid, after evaporation, a yellowish spot.

These reactions exclude all vegetable alkaloids save coniin. The putrefactive alkaloid does not distil when heated on the oil-bath to 200° , while coniin distils at 135° . The former is with certainty distinguished from coniin by its non-poisonous properties.

This substance is extracted by ether from acid, as well as from alkaline solutions. The yellow, oily drops obtained after the evaporation of the ether are soluble in alcohol. The taste is slightly burning.

SELM obtained from both putrefying and fresh intestines a

substance which gave the general alkaloidal reactions with potassium iodid, gold chlorid, platinum chlorid, potassio-mercuric iodid, and phospho-molybdic acid. It had strong reducing power, and when warmed with sulphuric acid gave a violet coloration. These reactions were not due to leucin, tyrosin, creatin, or creatinin. This is the substance which, as has been stated, SELMI considered identical with that observed by RÖRSCH and FASSBENDER and SCHWANERT. The minor differences observed by the different chemists may have been due to the varying degrees of purity in which the substance was obtained by them.

From human bodies from one to ten months after death, SELMI removed many alkaline bases. From an ether solution of a number of these, one was removed by treatment with carbonic acid gas. One base which was insoluble in ether, but readily soluble in amylie alcohol, was found to be a violent poison, producing in rabbits tetanus, marked dilatation of the pupils, paralysis, and death.

Parts of a human body preserved in alcohol were found by SELMI to yield an easily volatile, phosphorus-containing substance, which was soluble in ether and carbon disulphid, and gave a brown precipitate with silver nitrate. It was not the phosphid of hydrogen. A similar substance was produced by the slow decomposition of the yolks of eggs. With potassium hydrate it gave off ammonia and yielded a substance having an intense coniin odor. It was volatile and reduced phospho-molybdic acid.

SELMi also obtained from decomposing egg-albumin a body, the chlorid of which formed in needles, and possessed a curare-like action on frogs. From one arsenical body which had been buried for fourteen days, he obtained, by extracting from an alkaline (made alkaline with baryta) solution with ether, a substance which formed in needles and which gave crystalline salts with acids. With sulphuric acid it gave a red color; with iodic acid and sulphuric acid it liberated free iodine and gave a violet coloration; with nitric acid it gave a beautiful yellow, which deepened on the addition of caustic potash.

Platinum chlorid gave no precipitate save in highly concentrated solutions. From a second arsenical body, SELMI obtained by the same method a substance which gave, with tannic acid, a white precipitate; with iodine in hydriodic acid a kermes-brown; with gold chlorid a yellow, which was soon reduced; with mercuric chlorid a white; with picric acid a yellow, which gradually formed in crystalline tablets. This substance did not contain any arsenic, but was highly poisonous. From the stomach of a hog, which had been preserved in a solution of arsenious acid, SELMI separated an arsenical organic base. The fluid was distilled in a current of hydrogen. The distillate, which was found to be strongly alkaline, was neutralized with hydrochloric acid and evaporated to dryness when cross-shaped crystals, giving an odor similar to that of trimethylamin were obtained. This substance was found by CIACCIA to be highly poisonous, producing strychnia-like symptoms. With iodine in hydriodic acid it is said to give a gray, crystalline precipitate.

From the liquid which remained in the retort, a non-volatile arsenical ptomain was extracted with ether. An aqueous solution of this gave with tannic acid a slowly forming, yellowish precipitate, and similarly colored precipitates with iodine in hydriodic acid, platinum chlorid, auric chlorid, mercuric chlorid, potassio-mercuric iodid, potassio-bismuthic iodid, picric acid, and potassium bichromate. The physiologic action of this substance as demonstrated on frogs was unlike that of the arsines, but consisted of torpor and paralysis.

MORICCIA and BATTISTINI experimented with alkaloids, obtained from decomposing bodies, upon guinea-pigs and frogs, but did not attempt their isolation because of the rapid decomposition which they undergo when exposed to the air and by which they lose their poisonous properties. These alkaloids they found to be easily soluble in amylic alcohol, less soluble in ether.

In 1871 LOMBROSO showed that the extract from mouldy corn-meal produced tetanic convulsions in animals. (It must not be forgotten that similar effects may be due to the cornutin

of ergot.) This threw some light upon the cases of sporadic illness which had long been known to occur among the peasants of Lombardy, who eat fermented and mouldy corn-meal. In 1876 BRUGNATELLI and ZENONI obtained by the Stas-Otto method from this mouldy meal an alkaloidal substance which was white, non-crystalline, unstable, and insoluble in water, but readily soluble in alcohol and ether. With sulphuric acid and bichromate of potassium it yields a color reaction very similar to that of strychnin.

The action of the ether extracts from decomposed brain resembled that of curare, but was less marked and more transitory. The beats of the frog's heart were decreased in number and strengthened in force; the nerves and the muscles lost their irritability, and the animal passed into a condition of complete torpor. The pupils were dilated. GUARESCHI and MOSSO, using the Stas-Otto method, obtained from human brains which had been allowed to decompose at a temperature of from 10° to 15° for from one to two months, both volatile and non-volatile bases. Among the former only ammonia and trimethylamin were in sufficient quantity for identification. With these, however, were minute traces of ptomaines.

They obtained non-volatile bases from both acid and alkaline solutions. From the former they separated a substance which gave precipitates with gold chlorid, phosphotungstic acid, phospho-molybdic acid, Mayer's reagent, palladium chlorid, pieric acid, iodine in potassium iodid, and slightly with tannic acid. This substance was not precipitated with platinum or mercury.

From the alkaline extract there was obtained a substance which in dilute hydrochloric acid solutions gave with gold chlorid a heavy yellow precipitate with reduction, also precipitates with phospho-molybdic acid, platinum chlorid, Mayer's reagent, pieric acid, phosphotungstic acid, Marmé's reagent, iodine in potassium iodid, tannin, bichromate of potassium, palladium chlorid, and mercuric chlorid. It reduces ferric salts. From decomposed fibrin the same investigators obtained one well-defined ptomain. Analyses of the platinum

compound of this substance gave the formula $C_{10}H_{15}N$. This substance will be discussed in a future chapter.

From fresh brain substance they separated ammonia, trimethylamin, and an undetermined base. These, however, are not to be regarded as products of putrefaction, but as resulting from the action of the reagents upon the brain substance. The trimethylamin probably arises from the splitting up of lecithin, while the undetermined base is most likely cholin, which also results from the breaking up of the lecithin molecule.

They also show that when Dragendorff's method is used basic substances can be obtained from fresh meat, and these are shown to be produced by the action of the sulphuric acid on the flesh.

To BRIEGER (1882-88) is due the credit of isolating and determining the composition of a number of ptomaines. From putrid flesh he obtained neuridin, $C_5H_{14}N_2$, and neurin, $C_5H_{13}NO$. The former is inert, while the latter is poisonous. From decomposed fish he separated a poisonous base, $C_2H_4(NH_2)_2$, which is an isomer of ethylenediamin; muscarin, $C_3H_{15}NO_3$, and an inert substance, $C_7H_{17}NO_2$, gadinin. Rotten cheese yielded neuridin and trimethylamin. Decomposed glue gave neuridin, dimethylamin, and a muscarin-like base. In the cadaver, he has found in different stages of decomposition, cholin, neuridin, trimethylamin, cadaverin, $C_5H_{14}N_2$, putrescin, $C_4H_{12}N_2$, and saprin, $C_5H_{16}N_2$. These are all inert. After fourteen days of decomposition he found a poisonous substance, mydalein. From a cadaver which had been kept at from -9° to $+5^\circ$ for four months, BRIEGER obtained mydin, $C_8H_{11}NO$, the poisonous substance mydatoxin, $C_6H_{13}NO_2$, also the poison methyl-guanidin. From poisonous mussel he separated mytilotoxin, $C_6H_{15}NO_2$. From pure cultures of the typhoid bacillus of Koch and Eberth, BRIEGER obtained a poison, typhotoxin, and, from like cultures of the tetanus germ of Rosenbach and Nicolaier, tetanin. All of these bases will be discussed in detail in a subsequent chapter.

GAUTIER and ETARD isolated in 1881 the two bases which will be described later.

In 1885, VAUGHAN succeeded in isolating an active agent from poisonous cheese, to which he gave the name tyrotoxicon. This discovery has been confirmed by NEWTON, WALLACE, SCHAEFFER, STANTON, FIRTH, LADD, WOLFF, KIMURA, DAVIS, and KINNICUTT.

NICATI and RIETSCH, KOCH, and others, have shown the presence of a poisonous substance in cultures of the cholera bacillus. SALMON and SMITH have done the same with cultures of the hog-cholera germ; HOFFA with those of the anthrax bacillus; and BRIEGER with those of the tetanus germ.

In 1887, FODOR made his second contribution on the germicidal action of the blood *in vivo*. This gave an impetus to the further study of the germicidal properties of blood-serum and tissue-juices that has led to the most important results.

In 1888, CHRISTMAS obtained from cultures of the staphylococcus pyogenes aureus a substance which, when injected into the anterior chamber of the eye or under the skin, causes suppuration. ROUX and YERSIN showed that the chemie poison of Loeffler's diphtheria bacillus is a non-basic body which they believed to be of the nature of a ferment. NUTTALL showed that defibrinated blood has marked germicidal action.

In 1889, HANKIN isolated from cultures of the bacillus anthracis a poisonous albumose, which, when employed in large doses, proves fatal, and in small doses gives immunity. BUCHNER demonstrated that the germicidal properties of blood-serum remain after the destruction of all cellular elements.

In 1890, BRIEGER and FRAENKEL made their memorable contribution on bacterial poisons, in which they detail the methods by which they isolate their "toxalbumins" from cultures of the Loeffler bacillus, the anthrax bacillus, Eberth's germ, the cholera vibrio, and the staphylococcus pyogenes aureus. MARTIN made a more detailed study of the albumoses of anthrax. VAUGHAN reported toxins in cultures of

two toxicogenic germs found in drinking-water, also in cultures of three of Booker's summer diarrhoea germs and in poisonous cheese. NOVY and SCHWEINITZ found both basic and non-basic poisons in cultures of the hog-cholera bacillus. EHRLICH gave great impetus to the study of immunity by his researches on abrin and ricin. KOCH announced tuberculin as a cure for tuberculosis. Although the claims at first made for this discovery have not been realized, it must always be regarded as epoch-making in the history of medicine. OGATA and JASUHARA discovered that animals susceptible to anthrax might be made immune by treatment with the blood of animals naturally immune. This laid the foundation for the study of antitoxins. BEHRING and KITASATO showed that the blood of animals rendered immune artificially may protect susceptible animals, or that an antitoxin is formed during the process of securing artificial immunity.

In 1891, TIZZONI and CATTANI immunized animals to tetanus by gradually augmented doses of the toxin, and other animals with the blood-serum of the first. KITASATO ascertained that the trichlorid of iodine so attenuates the tetanus bacillus that it might be used without causing death and immunity could be secured in this way. BEHRING began his work on immunity against diphtheria in guinea-pigs.

In 1892, blood-serum therapy, which had been attempted before by BABES and TIZZONI, was brought into prominence by the researches of BEHRING. However, it was not until 1894 that serum was largely used in the treatment of diphtheria.

In 1893, one of the most important announcements was the discovery of the germicidal properties of the nucleins.

In 1894, the researches of PHILALIX and BERTRAND, and of CALMETTE on the venom of serpents and antivenomous serum are worthy of note as are those of METSCHNIKOFF on cholera, of ROUX on immunizing horses to diphtheria, and the general results obtained by the employment of blood-serum as a specific curative agent.

These and many other discoveries in the chemistry of bacteriology will be discussed in some detail in the following chapters.

CHAPTER III.

FOODS CONTAINING BACTERIAL POISONS.

POISONOUS MUSSELS.—Judging from the symptoms produced, there seem to be three different kinds of poisonous mussel. In one class the symptoms resemble those of a true gastro-intestinal irritant. FODERE reports the case of a sailor, who, after eating a large dish of mussels, suffered from nausea, vomiting, pain in the stomach, tenesmus, and rapid pulse. After death, which occurred within two days, the stomach and intestines were found inflamed and filled with a tenacious mucus. COMBE and others also report cases of the choleraic form of poisoning from mussel.

However, the symptoms which most frequently manifest themselves after the eating of poisonous mussels are more purely nervous. A sensation of heat and itching appears usually in the eyelids, and soon involves the whole face, and perhaps a large portion of the body. An eruption, usually called nettle-rash, though it may be papular or vesicular, covers the parts. The itching is most annoying, and may be accompanied by marked swelling. There follows a distressing asthmatic breathing, which is relieved by ether. In some cases reported by MOURING dyspnoea preceded the eruption, the patients became insensible, the face livid, and convulsive movements of the extremities were noticed. BURROW reports similar cases with delirium, convulsions, coma, and death within three days.

In a third class of cases there may be a kind of intoxication resembling somewhat that of alcohol, then paralysis, coma, and death.

In 1827, COMBE observed thirty persons poisoned, two of them fatally, with mussels. He described the symptoms as

follows: "None, so far as I know, complained of anything peculiar in the smell or taste of the animals, and none suffered immediately after taking them. In general, an hour or two elapsed, sometimes more; and the bad effects consisted rather in uneasy feelings and debility than in any distress referable to the stomach. Some children suffered from eating only two or three; and it will be remembered that Robertson, a young and healthy man, only took five or six. In two or three hours they complained of a slight tension at the stomach. One or two had cardialgia, nausea, and vomiting; but these were not general or lasting symptoms. They then complained of a prickly feeling in their hands, heat and constriction of the mouth and throat; difficulty of swallowing and speaking freely; numbness about the mouth, gradually extending to the arms, with great debility of the limbs. The degree of muscular debility varied a good deal, but was an invariable symptom. In some it merely prevented them from walking firmly, but in most of them it amounted to perfect inability to stand. While in bed they could move their limbs with tolerable freedom, but on being raised to the perpendicular posture they felt their limbs sink under them. Some complained of a bad, coppery taste in the mouth, but in general this was in answer to what lawyers call a leading question. There was slight pain of the abdomen, increased on pressure, particularly in the region of the bladder, which organ suffered variously in its functions. In some the secretion of urine was suspended, in others it was free, but passed with pain and great effort. The action of the heart was feeble; the breathing unaffected; the face pale, expressive of much anxiety; the surface rather cold; the mental faculties unimpaired. Unluckily, the two fatal cases were not seen by any medical person; and we are, therefore, unable to state minutely the train of symptoms. We ascertained that the woman, in whose house were five sufferers, went away as in a gentle sleep, and that a few moments before death she had spoken and swallowed."

The woman died within three hours, and the other death was that of a watchman, who was found dead in his box six

or seven hours after he had eaten the mussels. Postmortem examination in these showed no abnormality. The stomach contained some of the food partially digested.

The explorer VANCOUVER reports four cases similar to those observed by COMBE. One of the sailors died in five and a half hours after eating the mussels.

In some recent cases reported by SCHMIDTMANN, as quoted by BRIEGER, the symptoms were as follows: Some dock hands and their families ate of cooked blue mussels which had been taken near a newly-built dock. The symptoms appeared, according to the amount eaten, from soon after eating to several hours later. There was a sensation of constriction in the throat, mouth, and lips; the teeth were set on edge, as though sour apples had been eaten. There was dizziness, no headache; a sensation of flying, and an intoxication similar to that produced by alcohol. The pulse was hard, rapid (eighty to ninety), no elevation of temperature, the pupils dilated and reactionless. Speech was difficult, broken, and jerky. The limbs felt heavy; the hands grasped spasmodically at objects and missed their aim. The legs were no longer able to support the body, and the knees knocked together. There was nausea, vomiting, no abdominal pain, no diarrhœa. The hands became numb and the feet cold. The sensation of cold soon extended over the entire body, and in some the perspiration flowed freely. There was a feeling of suffocation, then a restful and dreamless sleep. One person died in one and three-quarters of an hour, another in three and one-half hours, and a third in five hours, after eating of the mussels.

In one of these fatal cases rigor mortis was marked and remained for twenty-four hours. The vessels of all the organs were distended, only the heart was empty. VIRCHOW concluded from the conditions observed that the blood had absorbed oxygen with great avidity. There was marked hyperemia and swelling of the mucous membrane of the stomach and intestines, which Virchow pronounced an enteritis. The spleen was enormously enlarged and the liver showed numerous hemorrhagic infarctions.

Many theories have been advanced to account for poisonous mussels. It was formerly believed that the effects were due to copper which the animals obtained from the bottoms of vessels; but, as CHRISTISON remarks, copper does not produce these symptoms. Moreover, CHRISTISON made analysis of the mussels which produced the symptoms observed by COMBE, and was unable to detect any copper. BOUCHARDAT found copper in some poisonous mussels, but he does not state the amount of the copper nor the source of the animals.

EDWARDS advanced the theory that the symptoms were wholly due to idiosyncrasy in the consumer. This may be true in some instances where only one or two of those partaking of the food are affected, but it certainly is not a tenable hypothesis in such instances as those reported by COMBE and SCHMIDTMANN, where a large number or all those who partook of the food were affected.

COLDSTREAM found the livers of the Leith mussels, as he thought, larger, darker, and more brittle than normal, and to this diseased condition he attributed the ill effects.

LAMOROUX, MOHRING, DE BEUME, CHENU, and DU RONDEAU have supposed that the poisonous effects were due to a particular species of meduse upon which the mussels feed. DE BEUME found in the vomited matter of one person, suffering from mussel poisoning, some medusæ, and he states that these are most abundant during the summer, when mussels are most frequently found to be poisonous.

The theory of BURROW that the animal is always poisonous during the period of reproduction has been received with considerable credit. However, cases of poisoning have occurred at different seasons of the year.

CRUMPE, in 1872, suggested that there is a species of mussel which is in and of itself poisonous, and this species is often mixed with the edible variety. SCHMIDTMANN and VIREHOW support this idea. They state that the poisonous species has a brighter shell, a sweeter, more penetrating, bouillon-like odor than the edible kind, also that the flesh of the former is yellow and that the water in which they are cooked is bluish.

LOHMEYER also champions this opinion. This theory, however, is opposed by the majority of zoologists. MÖBIUS states that the peculiarities of the supposed poisonous variety pointed out by VIRCHOW and SCHMIDTMANN are really due to the conditions under which the animal lives, the amount of salt in the water, the temperature of the water, whether it is moving or still water, the nature of the bottom, etc. Finally, MÖBIUS states that the sexual glands, which form the greater part of the mantle, are white in the male and yellow in the female. However, it has been shown later by SCHMIDTMANN and VIRCHOW that edible mussels may become poisonous if left in filthy water for fourteen days or longer, and, on the other hand, poisonous ones may become fit for food if kept for four weeks in good water.

Cats and dogs which have eaten voluntarily of poisonous mussels have suffered from symptoms similar to those observed in man; and rabbits have been poisoned by the administration of the water in which the food has been cooked. A rabbit which was treated in this manner by SCHMIDTMANN died within one minute. From these mussels BRUEGER extracted the ptomain mytilotoxin, which will be discussed in a subsequent chapter. This poison has a curare-like action. Whether or not those mussels which produce other symptoms also contain ptomains, remains for future investigations to determine.

In 1887 three other cases of mussel poisoning, one fatal case, occurred at Wilhelmshaven, the place which supplied BRUEGER with the mussels from which he obtained mytilotoxin. SCHMIDTMANN has found that non-poisonous mussels placed in the waters of this bay soon become poisonous, and that the poisonous mussels from the bay placed in the open sea soon lose their poisonous properties. LINDER has found in the water of the bay and in the mussels living in it a great variety of protozoa, amœba, bacteria, and other lower organisms, which are not found in the water of the open sea nor in the non-poisonous mussel. He has also found that, if the water of the bay be filtered, non-poisonous mussels in it do not become poisonous. He therefore concludes that poisonous mus-

sels are those which are suffering from disease due to residence in filthy water.

CAMERON examined mussels that poisoned seven persons, five fatally, near Dublin. The symptoms were vomiting, dyspnœa, incoordination, and spasms. The mussels contained enlarged livers; and a base, probably identical with mytilotoxin, was isolated. The water in which these mussels lived was contaminated with sewage.

BRIEGER has tested dead and decomposed mussels taken from the open sea for mytilotoxin, with negative results.

POISONOUS OYSTERS AND EELS.—PASQUIER reported cases of poisoning at Havre from the eating of oysters taken from an artificial bed which had been established near the outlet of a drain from a public water-closet. CHRISTISON says that an "unusual prevalence of colic, diarrhœa, and cholera" at Dunkirk was believed to have been traced to an importation of unwholesome oysters from the Normandy coast. VAUGHAN and NOVY obtained tests for tyrotoxin in the liquor of some decomposed oysters which had caused illness in many people at a church festival.

VIREY states that many persons were attacked with violent pain and diarrhœa a few hours after eating a paté made of eels from a stagnant cattle-ditch near Orleans, also that similar cases have occurred in various parts of France, and that domestic animals have been killed by eating the remains of the poisonous dish.

The uncooked blood and the blood-serum of the common river eel, the Conger eel, and the murena are highly poisonous. PENNAVARIA reports the case of a man who mixed the blood from 0.64 kilo. of eel with 200 c.c. of wine and drank it. A most violent purging, accompanied by insalivation, stertorous breathing, leaden countenance, and glassy expression of the eyes, resulted. Recovery followed the administration of opium. Mosso has named the poisonous constituent of eel serum ichthyotoxinum. It burns the tongue like phosphorus. Subcutaneously it causes marked local reaction.

For experimental purposes it is best administered intravenously. It induces in dogs convulsions, but not constantly. The respiration is at first greatly accelerated and then ceases. By artificial respiration, life can be prolonged. The pupils are dilated, though they may contract before death. Blood pressure is at first greatly increased, then it falls rapidly. The blood loses its property of coagulation. When heated to 100° the poison becomes inert and loses its sharp, burning taste. Acetic and mineral acids destroy it. Mosso concludes that ichthyotoxium is a serum-albumin and finds that it can be precipitated from the serum, and still retain its poisonous properties by saturation with ammonium sulphate. SPRINGFIELD finds that the blood-serum of the river eel induces at first nervous excitement, then convulsions followed by paralysis. His work confirms that of Mosso.

FISH POISONING.—Some fish are always poisonous. Others are poisonous, or, at least, markedly so, only during the spawning season. Still others are subject to epidemic bacterial diseases, and those affected with certain of these diseases furnish flesh that is toxic to man, or, in other words, the bacterial disease is transmitted to man with this food. Lastly, fish, like other kinds of meat, may become infected with saprophytic germs that may harm man. The Spaniards use the word *signatera*¹ to designate the complex of symptoms induced in man by the eating of fish that are physiologically poisonous; and BLANCHARD proposes the general adoption of this term, while he suggests that the word *botulismus* or *botulism* be used to designate diseased conditions which result from the eating of any kind of meat that is harmful on account of bacterial infection. He says substantially as follows:

“There are two distinct categories of intoxication with the flesh of vertebrates:

“*Botulismus* is an intoxication induced by meat invaded by microbes and the ptomaines elaborated by them. This

¹ Pronounced sig-wah-té-ra.

term is applicable not only to disease caused by market meat, but also to that induced by preserved foods.

"Siguatera is an intoxication caused by fresh food, not infected with bacteria, and in which the poisonous principles are leucomains formed by the physiological activity of the tissues. I propose to designate this category of intoxication by the word *siguatera*, a name employed by the Spanish physicians of the Antilles to indicate poisoning by eating fish."

ROBERT makes the following classification of poisonous fish :

1. Many fish possess poisonous glands that are connected with their barbed fins with which they wound their enemies. The structure of these glands is similar to those of poisonous snakes. After the removal of the skin containing these glands the flesh is not poisonous. Such are *Trachinus draco* of the German lakes and *Serranus scriba* of the Mediterranean Sea. *Stomias boa* is feared on account of its bite ; many roaches have a poisonous barb in the tail. BOTTARD describes five classes of fish supplied with poison-glands : 1. Of this class, *Synanceia brachio* is a type and has its poison apparatus in the dorsal fin, consisting of thirteen barbs, each of which has two poison reservoirs. Each of the twenty-six reservoirs is supplied by ten or twelve tubular glands, the secretion of which is a clear, bluish, feebly acid fluid. Undiluted this secretion causes local gangrene ; when diluted it causes paralysis. In *Plotosus lanceatus* in front of the ventral fin there is a hollow barb with closed end, connected with a poison reservoir, and the fluid flows only when the barb is broken. 2. *Trachinus draco* is a typical example of this class. The apparatus of *Cottus scorpio* and *C. bubalis* also is of this kind. There are three hollow barbs on the gill cover and the reservoirs connected with them secrete a poison only during the spawning season. 3. *Thalassophryne reticulata* has two hollow barbs, one on the gill cover and the other on the back. 4. *Murena helena* has on the gums an open pocket, the walls of which are lined with cells secreting a

poison that moistens the teeth. 5. *Scorpena scropha* and *S. porcus* have open poison-glands connected with hollow barbs situated in the dorsal and caudal fins. Chemically nothing is known of the nature of these poisons; pharmacologically it has been demonstrated that they cause severe inflammation of the subcutaneous tissue.

2. The fish poisoning so well known in Japan is due to different species of *Tetrodon* (Fugu). According to REMY there are in Japan twelve species of fish, all belonging to the genus *Tetrodon*, whose ovaries are poisonous. In winter when the ovaries are atrophied they are least harmful. However, REMY reports the following experiments made with fish caught during the winter:

Dogs fed upon the ovaries or testicles soon sickened, with salivation, severe and frequent vomiting and convulsive muscular contractions. Soon after the poison was gotten out of the stomach by vomiting recovery followed. In order to prevent this rapid elimination the organs were rubbed up in a mortar and the fluid portion administered subcutaneously. By this method, notwithstanding the fact that the experiments were made in winter, death resulted in less than two hours. The symptoms consisted chiefly of disturbances of the digestive and nervous systems. The most constant were uneasiness, salivation, vomiting of much mucus, severe contractions of the abdomen, then paralytic symptoms, relaxation of the sphincters, marked dyspnoea, cyanosis and dilatation of the pupils. Death was due to dyspnoea. On section, the salivary glands and pancreas were found injected and ecchymosed. There were small hemorrhagic spots in the stomach and intestines. The liver and kidneys were filled with dark blood as is seen in death from asphyxiation. No structural changes could be found in the nervous system.

In men the first and most prominent symptoms are referable to the nervous system, although the vomiting may be so severe that gastric hemorrhage occurs.

3. *Clupea thrissa* and *C. venenosa*, also certain species of *Scarus*, have no poisonous glands, nor are their reproductive

organs more poisonous than other parts of the body. Still these fish are always poisonous. According to GUNTHER their harmful properties are due to the medusæ, corals, and other decomposing substances upon which they feed. In the West Indies it is a recognized fact that all the fish caught off certain coral banks are poisonous. Every part of the animal is unfit for food. The symptoms are those of a gastro-enteritis and death frequently results.

4. In this class are included the cases that BLANCHARD would describe under botulism. The poison is due to putrefactive changes. Instances of this will be mentioned later.

5. In Russia many instances of fish-poisoning are due to the fact that the fish are diseased and the disease is transmitted to man in his food. The most prominent symptoms are cerebro-spinal. Instances of this kind of fish-poisoning are well known in Germany also, and here they are due to eating diseased barbels. The symptoms are identical with those of cholera nostras, and the disease is known as "barbencholera." The poison, the nature of which is yet unknown, evidently severely irritates the mucous membrane of the stomach and intestines. This form of fish-poisoning is sometimes called *ichthyismus gastricus*. The fish, *Schistothorax*, found in the rivers of middle Asia, has a similar effect. *Ichthyismus exanthematicus*, in which, along with or following the gastrointestinal irritation, there is a scarlatinous rash, is probably also due to the eating of diseased fish. Gar eels, oysters, mussels, and certain species of mackerel are especially likely to induce this disease.

Petromyzon fluviatilis, which is not classed among fish by modern zoologists, causes, according to PROKHOROW, a bloody diarrhoea very frequently in the Jamberg district of Russia. This occurs whether the fish is eaten raw or thoroughly cooked. It is stated that if salt be sprinkled on the animal while yet alive its skin secretes an abundant discharge of mucus and after this the flesh is not poisonous.

BÖHM and others have expressed some doubt about any species of fish being *per se* poisonous. They have been in-

clined to attribute the effects so frequently observed to one or the other of the following causes: 1. The meat rapidly undergoes putrefactive changes and the ill effects are due to true botulism. 2. The observed untoward symptoms are explainable by supposing the existence of a marked idiosyncrasy in the consumer. That the first supposition is not true is shown by the following facts: 1. Poisoning with perfectly fresh fish occurs not only in the tropics, where decomposition goes on rapidly, but in the temperate zone as well, and during seasons of the year and under conditions that exclude the possibility of the ill effects being due to putrefactive changes in the meat. 2. Certain species of tetrodon and other fish are so well known to be poisonous, even when perfectly fresh, that their consumption is at times resorted to, notably in China and Japan, for suicidal purposes.

That the symptoms are not due to idiosyncrasy in the consumer is demonstrated by the effects of the flesh and of the expressed juices upon the lower animals.

It has also been suggested that the poisoning may be due to substances of vegetable origin which are employed in some countries, notably by savage and partly civilized peoples, to kill the fish. That this may have been true in some instances is possible, but that this explanation is not applicable to any large number of instances is shown by the observation that where this method of obtaining fish for food is most frequently practised no ill results follow, and where it is not resorted to cases of fish-poisoning may be very common. According to HUSEMANN, *Cocculus indicus* has been employed for the purpose of catching fish. The leguminous plant, *Piscidia*, of the West Indies owes its name to this use of its bark. In the Dutch East Indies the cortex of the root of *Derris elliptica* and the seed of *Pachyrrhizus angulatus* are employed for this purpose. Both of these, according to GRESHAM, contain a non-nitrogenous substance which is highly poisonous to fish, and relatively harmless to other animals. An extract of the derris root, which, in Borneo, is also used as an arrow poison, kills fish when mixed with the water in the proportion of

1 : 25,000. and the active principle in a dilution of 1 : 5,000,000. GRESHOF has isolated both of these poisons and named them derrid and pachyrrhizid. A legumin, *Tephrosia ichthyonecea*, from West Africa also yields a non-nitrogenous poison, but this affects other animals as well as fish. The fish-poison of Java, from the seed of *Milletia atropurpurea*, contains saponin, and that of Ceylon, from *Hydrocarpos inebrians*, owes its effects to hydrocyanic acid. (HUSEMANN.) *Robinia nicon* of tropical America is used by the savage tribes for the purpose of benumbing fish. GEOFFREY and SCHLAGDENHAUFFEN found this plant to contain a snow-white, crystalline substance, freely soluble in alcohol, wholly insoluble in water. Water, to which an alcoholic solution of this poison had been added in the proportion, 1 : 1,000,000, killed fish. Other fish-poisons of the West Indies are *Jacquinia armillaris*, which, on account of the fact that its dried fruit is used for bracelets, is known as *bois bracelet*, and *Serjania letalis*, from which the poisonous honey of a certain wasp is prepared, the toxic action of which ST. HILAIRE tested upon himself. (HUSEMANN.)

SCHMIDT concludes his studies on some poisonous fish in Russia with the following statements :

1. Poisoning with fish is not due to putrefaction.
2. Fish-poisoning (in Russia) is always due to some member of the sturgeon tribe.
3. The genesis of fish-poisoning has no relation to the method of catching the fish, the use of salt, or imperfections in the methods of preserving.
4. The poisonous substance is not distributed throughout the animal, but is confined to certain parts.
5. The poisonous portion cannot be distinguished from the non-poisonous, either macroscopically or microscopically.
6. The (thoroughly) cooked meat is never poisonous.
7. The fish-poison is an animal alkaloid, produced most probably by bacteria that cause an infectious disease in the fish *intra vitam*.

ARUSTAMOW has studied eleven cases of fish-poisoning with

five fatal cases. Some were caused by eating sturgeon and others were due to salmon. In the fish, and in the liver, kidneys, and spleen of the dead persons, germs, resembling but not identical with the typhoid bacillus, were found. Moreover, the bacilli from the sturgeon and those from the salmon were not identical, the latter being both thicker and longer than the former. The most noteworthy symptoms were general weakness, dull pain in the abdomen, dyspnoea, mydriasis, vertigo, and dryness of the mouth. This author also concludes that the ill effects are due to bacteria, which are pathogenic to the fish. The fish was eaten raw.

According to ANREP there are in poisonous fish two poisonous ptomaines. One of these is extracted from alkaline solution with ether, chloroform, and benzin. It is amorphous and insoluble in water, but forms easily soluble salts of great toxicity, so that one-fourth milligram of the hydrochlorid produces poisonous effects in dogs, and one-half milligram kills rabbits. This ptomain may be preserved quite indefinitely in the dry state or dissolved in ether, but is speedily destroyed by strong alkalis and acids. Dissolved in phosphoric acid and evaporated it gives a red coloration, rapidly passing into a dirty green. It gives precipitates with iodine in potassium iodid, potassio-bismuth iodid, potassio-cadmie iodid, phosphotungstic acid, and pierie acid. With potassium ferri-cyanid and ferric chlorid, it gives a blue coloration only after many hours. JAKOLEW isolated a similar alkaloid from poisonous sturgeon in 1889, but it differed from that of ANREP in the fact that the former gave precipitates with platinum chlorid and tannin, while the latter did not. ANREP's second ptomain is an oily substance, less poisonous than the solid.

These ptomaines have a paralyzing action on frogs, dogs, and rabbits, arresting respiration and the action of the heart. In cats there are chronic convulsions. The heart's action is retarded, and just before death the respiration is accelerated. The more poisonous of these alkaloids, for which the name halichthytoxin has been suggested, produces mydriasis on local application to the eye.

Halichthytoxin is destroyed by boiling, and this suggests a possible remedy against fish-poisoning, in Russia, by thorough cooking. In Astrachan a woman was rendered seriously ill by eating a small bit of raw sturgeon, while others ate portions of the same fish, thoroughly cooked, without ill effects.

TAKAHASCHI and INOKO find the fugu poison of the tetrodon highly resistant to prolonged boiling. This poison also differs radically in its solubility and its behavior to alkaloidal reagents from that of the fish of Russia. It is freely soluble in water and is not precipitated by lead acetate, normal or basic, nor by corrosive sublimate, phosphotungstic acid or platinum chlorid. In two cases of fatal poisoning with tetrodon TAKAHASCHI and INOKO have induced the characteristic symptoms of fugu poisoning by injecting the blood, urine, and aqueous contents of the stomach into the abdominal cavity of frogs.

STEVENSON reports a case of poisoning from eating canned sardines. The victim, a strong, healthy man, died apparently of malignant œdema twenty-five hours after partaking of this food. Bits of the liver introduced under the skin of guinea-pigs caused malignant œdema. Notwithstanding these facts no pathogenic organism could be found either in the organs or vomit of the dead man or in the pigs. The presence of a highly poisonous crystalline ptomain is reported. It is probable that the failure to find the germ was due to the neglect of attempting to grow it in anaerobic culture.

GRESSIN states that there is no poison-gland connected with the barb on the gill covering of *Trachinus draco* (weever), but that the pocket, in which the opercular fin lies, is lined with large epithelial cells, which probably secrete the poison. This substance kills small fish, frogs, and rats, in which convulsions and fall of temperature precede death. One drop of the fluid injected subcutaneously in pigeons causes convulsive trembling and spasmodic breathing. While GRESSIN found that the poison of the weever fish at Havre induces convulsions in frogs, POUL found that the poison of the same fish from the Adriatic, also that of *Trachinus radiatus*, acts as an exquisite heart poison, retarding and finally arresting this

organ in diastole. Its action on the heart is not altered by atropin, camphor, caffenin, helleborein or hydrastin. Along with its effects on the heart, spontaneous movement and cutaneous sensibility are impaired. A similar, though less active, poison is found in the barb of the dorsal fin. The small immovable barbs in the caudal fin of *Scorpoena porcus* (hog fish), so much dreaded by fishermen, are supplied with an analogous but less active poison. Neither the blood-serum nor the raw flesh of the trachinus has poisonous properties.

VAUGHAN reports the following case of ichthyism exanthematicus from eating canned salmon: K., a very vigorous man of 34 years, ate freely of canned salmon. Others at the table with him remarked that the taste of the salmon was peculiar and refrained from eating it. Twelve hours later K. began to suffer from nausea, vomiting, and a griping pain in the abdomen. Eighteen hours after he had eaten the fish the writer saw him. He was vomiting small quantities of mucus, colored with bile, at frequent intervals. The bowels had not moved and the griping pain continued. He was covered with a scarlatinous rash from head to foot. His pulse was 140, temperature 102° F., and respiration shallow and irregular. The stomach and large intestines were washed out thoroughly, and ten grains of calomel, soon followed by twelve ounces of solution of magnesium citrate, for the purpose of cleansing the small intestines, were administered. After these medicines had acted freely K. began to improve. The next day the rash disappeared, but the temperature remained above the normal for four or five days, and it was not until a week later that the man was able to leave his house. The remainder of the salmon was submitted to various tests. The absence of inorganic poisons was demonstrated. It was found that the subcutaneous injection of twenty drops of the fluid expressed from the salmon caused evident illness and suffering in a white rat. The only germ that could be found, either by direct microscopic examination or by the preparation of plate cultures, was a micrococcus, and this was present in the salmon in great numbers. This germ grew fairly well in beef-tea, but

the injection of five c.c. of the beef-tea cultures of different ages failed to affect white rats, kittens, or rabbits. However, this micrococcus when grown for twenty days in a sterilized egg, after Hueppe's method of anaerobic culture, produced a most potent poison. The white of the egg became thin, watery, markedly alkaline, and ten drops sufficed to kill white rats.

MIURA and TAKESAKI find that the ripe ovaries of *tetrodon rubripes* contain a substance which induces in rabbits acceleration of the respiratory movements, paralysis of the skeletal muscles, mydriasis, increased peristalsis of the intestines, and arrest of the heart.

The disease known as "kakke," which prevails from May to October in Tokio, is, according to MIURA and others, an intoxication due to the eating of fish, which belongs to the *scombridae*. The affection is generally chronic or subacute, seldom acute. The most characteristic symptom is paralysis of the diaphragm with consequent dyspnea and disturbance of the action of the heart. Electrical stimulation of the diaphragm has proven to be the most successful treatment.

GRIFFITHS found in sardines that had undergone putrefactive changes a base to which he has given the name sardinin. This ptomain, together with others reported by GRIFFITHS, is described in Chapter XII.

POTAIN saw a man suffering from vomiting, vertigo, ringing in the ears, and pain in the joints due to eating lobster. There was pain in the bowels, but no stool.

SIEBER has recently examined into the cause of a fish epidemic in Russia. In an aquarium, from which fish were taken to supply a castle table, as many as thirty dead fish were found in the course of two days. From the dead and sick fish and from the aquarium water SIEBER obtained by anaerobic methods a highly toxicogenic germ to which she has given the name *bacillus piscicidus agilis*. This bacillus consists of highly motile short rods. Old cultures, especially those in bouillon, have spores. The bacilli are easily colored with Ziehl's solution. On gelatin and agar plates the colonies are granular, gray or

yellow, and liquefy gelatin, each colony consists of three concentric zones, the outer one being indented. Stich cultures in gelatin or agar grow at from 12° to 37.5° , producing carbonic acid gas and small quantities of methylmercaptan. On potatoes, the growth consists of yellowish or brownish glistening spots. Milk is coagulated. *Bacillus piscicidus agilis* retains its virulence for months, but does not multiply in well and river water. It is destroyed by a temperature of from 60° to 65° . The older the culture the more poisonous it is. Gelatin cultures are most virulent.

Bacillus piscicidus agilis is pathogenic to fish, frogs, mice, rabbits, dogs, and guinea-pigs. From the muscles of these animals the germ may be recovered in pure culture. Cultures filtered through a Chamberland bougie are as poisonous as the unfiltered ones. In the distillate from cultures there is a poison. Filtered cultures give an intense red coloration with ferric chlorid. SIEBER has obtained from cultures of this bacillus cadaverin and other known ptomaines, but there are, at least, two more bases present. Three and one-half milligrams of one of these suffice to kill a frog in fifteen minutes.

The symptoms induced in the animals by the use of sterilized cultures consist of shortness of breath and unrest, followed by apathy and paralysis.

SAUSAGE POISONING.—This is also known as botulismus and allantiasis. While considerable diversity has been observed in symptoms of sausage poisoning, we cannot divide the cases into classes from their symptomatology as has been done in mussel poisoning. The first effects may manifest themselves at any time from one hour to twenty-four hours after eating of the sausage, and cases are recorded in which it is stated no symptoms appeared until several days had passed. However, we must remember that trichiniasis was frequently, in former times, classed as sausage poisoning, and it is highly probable that these cases of long delay in the appearance of the symptoms were really not due to putrefaction, but to the presence of parasites in the meat. A large

majority of the one hundred and twenty-four cases more recently reported by MÜLLER sickened within twenty-four hours, and out of the forty-eight of these that were fatal, six died within the first twenty-four hours. At first there is dryness of the mouth, constriction of the throat, uneasiness in the stomach, nausea, vomiting, vertigo, indistinctness of vision, dilatation of the pupils, difficulty in swallowing, and usually diarrhœa, though obstinate constipation may exist from the first. There is, as a rule, a sensation of suffocation, and the breathing becomes labored. The pulse is small, thready, and rapid. In some cases the radial pulse may be imperceptible. Marked nervous prostration and muscular debility follow. These symptoms vary greatly in prominence in individual cases. The retching and vomiting, which may be most distressing and persistent in some instances, in others are trivial at the beginning and soon cease altogether. The same is true of the diarrhœa. As a rule, the functions of the brain proceed normally, but there may be delirium, then coma and death. In some there are marked convulsive movements, especially of the limbs, in others paralysis may be an early and marked symptom. The pupils may dilate, then become normal and again dilate. There is frequently ptosis, and paralysis of the muscles of accommodation is not rare. Complete blindness has followed in a few instances.

The fatality varies greatly in different outbreaks. In 1820 KERNER collected reports of seventy-six cases, of which thirty-seven were fatal. In his next publication (1822) he increased the number to one hundred and fifty-five cases, with eighty-four fatal results. This gave a mortality of over fifty per cent., while in one outbreak reported by MÜLLER the mortality was less than two per cent.

A large proportion of the cases of sausage poisoning have occurred in Württemberg and the immediately adjacent portions of Baden. This fact has, without doubt, been correctly ascribed to the methods there practised of preparing and curing the sausage. It is said to be common for the people to use the blood of the sheep, ox, and goat in the preparation

of this article of diet. Moreover, the blood is kept sometimes for days in wooden boxes and at a high temperature before it is used. In these cases it is altogether likely that putrefaction progresses to the poisonous stage before the process of curing is begun. However, cases of poisoning have occurred from beef and pork sausages as well.

Moreover, the method of curing employed in Würtemberg favors putrefaction. A kind of sausage known as "blunzen" is made by filling the stomachs of hogs with the meat. In curing, the interior of this great mass is not acted upon, and putrefaction sets in. The curing is usually done by hanging the sausage in the chimney. At night the fire often goes out and the meat freezes. The alternate freezing and thawing render decomposition more easy. The interior of the sausage is generally the most poisonous. Indeed, in many instances those who have eaten of the outer portion have been unharmed, while those who have eaten of the interior of the same sausage have been most seriously affected.

The above-mentioned methods of preparing sausage in Würtemberg are now not so generally employed, and poisoning from this article of food is not so common as formerly.

Many German writers state that when a poisonous sausage is cut, the putrid portion has a dirty, grayish-green color, and a soft, smeary consistency. A disagreeable odor, resembling that of putrid cheese, is perceptible. The taste is unpleasant, and sometimes a smarting of the mouth and throat is produced. Postmortem examination after sausage poisoning shows no characteristic lesion. It is generally stated that putrefaction sets in very tardily, but MÜLLER shows that no reliance can be placed upon this point, and states that out of forty-eight recorded autopsies, it was especially stated in eleven that putrefaction rapidly developed. In some instances there has been noticed hyperemia of the stomach and intestinal canal, but this is by no means constant. The liver and brain have been reported as congested, but this would result from the failure of the heart, and would, by no means, be characteristic of poisoning with sausage.

VON FABER, in 1821, observed sixteen persons who were made sick by eating fresh, unsmoked sausage made from the flesh of a pig which had suffered from an abscess on the neck. Five of the patients died. The symptoms were as follows: There was constriction of the throat, difficulty in swallowing, retching, vomiting, colic-like pains, vertigo, hoarseness, dimness of vision, and headache. Later and in severer cases, there was complete exhaustion, and, finally, paralysis. The eyeballs were retracted, the pupils were sometimes dilated, then contracted; they did not respond to light; there was paralysis of the upper lids. The tonsils were swollen, but not as in tonsillitis. Liquids which were not irritating could be carried as far as the œsophagus, when they were then ejected from the mouth and nose with coughing. Solid foods could not be swallowed. On the back of the tongue and in the pharynx there was observed a puriform exudate.

Obstinate constipation existed in all, while the sphincter ani was paralyzed. The breathing was easy, but all had a croupous cough. The skin was dry. There was incontinence of urine. There was no delirium and the mind remained clear to the last.

Postmortem examinations were held on four. The skin was rough—"goose-skin." The abdomen was retracted. The large vessels in the upper part of the stomach were filled with black blood. The contents of the stomach consisted of a reddish-brown, semi-fluid substance, which gave off a repugnant, acid odor. In one case the omentum was found greatly congested. The large intestine was very pale, and the right ventricle of the heart was filled with dark fluid blood.

Scnüz cites thirteen cases of poisoning from liver sausage in which the symptoms differed from the foregoing in the following respects:

1. In only one out of the thirteen was there constipation; all the others had numerous watery, typhoid-like stools.
2. Symptoms involving the sense of sight were present in only three; in all the pupils were unchanged.

3. The croupous cough was wholly wanting; though in many there was complete loss of voice. Difficulty of swallowing was complained of by only one.

4. Delirium was marked in all; and in one the disturbance of the mental faculties was prominent for several weeks.

5. There were no deaths.

6. The time between eating the sausage and the appearance of the symptoms varied from eighteen to twenty-four hours, and the duration of sickness from one to four weeks; though in one case complete recovery did not occur until after two and one-half months.

The sausages were not smoked, and all observed a garlic odor, though no garlic had been added to the meat.

TRIPE reports sixty-four cases. The symptoms came on from three and one-half to thirty-six hours after eating. The stools were frequent, watery, and of offensive odor. In some there was delirium. One died. In the fatal case the hands and face were cold and swollen. The pulse was rapid and weak. The pupils were contracted, but responded to light. The small intestine was found inflamed.

HEDINGER reports the case of a man and a woman with the usual symptoms, but during recovery the dilatation of the pupils was followed by contraction. Birds ate of this sausage, and were not affected.

RÖSER reports cases in which there were found, after death, abscesses of the tonsils, a dark, bluish appearance of the mucous membrane of the pharynx, larynx, and bronchial tubes, dark redness of the fundus of the stomach, and circumscribed, gray, red, and black spots on the mucous membrane of the intestine. The liver was brittle and the spleen enlarged.

Many theories concerning the nature of the active principle of poisonous sausage have been advanced. It was once believed to consist of pyroligneous acid, which was supposed to be absorbed by the meat from the smoke used in curing; but it was soon found that unsmoked sausage might be poisonous also. EMMERT believed that the active agent was hydrocyanic acid, and JÄGER's theory supposed the presence of

picric acid. But these acids are not found in poisonous sausage, and, moreover, their toxicologic effects are wholly unlike those observed in sausage poisoning. As we have elsewhere seen, KERNER believed that he had found the poisonous principle in a fatty acid. This theory was supported by DANN, BUCHNER, and SCHUMANN. KERNER believed the poison to consist of either caseic or sebacic acid, or both, while BUCHNER named it *acidum botulinicum*; but the acids of the former proved to be inert, and that of the latter to have no existence. SCHLOSSBERGER first suggested that the poisonous substance is most probably basic in character, and he found an odoriferous, ammoniacal base which could not be found in good sausage, and which did not correspond to any known amides, imides, or nitril bases. However, this substance has not been obtained by anyone else, nor has it been demonstrated to be poisonous.

LIEBIG, DUFLAS, HIRSCH, and SIMON believed in the presence of a poisonous ferment. VAN DEN CORPUT described *sarcina botulina*, which was believed to constitute the active agent. MÜLLER, HOPPE-SEYLER, and others have found various microorganisms, and VIRCHOW, EICHENBERG, and others have examined microscopically the blood of persons poisoned with sausage. Recently, EURENBERG has attempted to isolate the poisonous substance by employing Brieger's method, but he obtained only inert substances.

GAFFKY and PAAK have made a thorough study of some sausage which poisoned a large number of people, among whom one, a strong man, died. The sausage was made of horse-flesh and liver. In the majority of the persons the symptoms came on within six hours and in one instance within half an hour. Many had a severe chill; some did not. The most prominent symptoms were headache, loss of appetite, pain in the bowels, vomiting and purging. In the fatal case, however, there was no vomiting. From the sausage GAFFKY and PAAK isolated a short bacillus, which, when given by the mouth, subcutaneously or intravenously, produced the above symptoms, with a fatal termination in most instances,

in rabbits, guinea-pigs, mice, and apes. GAFFKY and PAAK were unable to isolate the chemical poison.

This bacillus has only a rotatory motion and does not change its location. Frequently it stains only at the poles. The colonies on gelatin are always small, not larger than pin-heads. It is quite resistant to high temperature, requiring fifteen minutes at from 75° to 80° to destroy it.

POISONOUS MEAT.—Under this head we shall not discuss cases of poisoning from trichina or other parasites, but shall refer only to those instances in which the toxic agent has originated in putrefactive changes. A number of such cases have been observed in the past fifteen years, but only a few of them have been investigated scientifically. The best known of these will be briefly discussed.

In June, 1880, a large number of persons attended a sale of timber and machinery on the estate of the Duke of Portland at Wellbeck. The sale continued four days, and lunches were served by the proprietress of a neighboring hotel. The refreshments consisted of cold boiled ham, cold, boiled or roasted beef, cold beefsteak pie, mustard and salt, bread and cheese, pickles, and Clumney sauce. The drinks were bottle and draught beer, spirits, ginger beer, lemonade, and water. Many were poisoned, and BALLARD obtained the particulars of seventy-two cases, among which there were four deaths. The symptoms are given by BALLARD as follows:

“I propose to speak of the attacks under the name of ‘diarrhœal illness,’ because diarrhœa was the most constant of all the symptoms observed, and the other symptoms were in some respects so peculiar that I am indisposed to give to the disease any name otherwise generally recognized. As might have been anticipated from our experience of diseases in general, there were varieties in severity among the cases investigated; and symptoms strongly marked in some, were slightly marked or altogether wanting in others. Perhaps I shall do the best service by giving first a general sketch of the course

of the illness, subsequently illustrating it by a description of a few well-marked cases.

"A period of incubation preceded the illness. In fifty-one cases where this could be accurately determined, it was twelve hours or less in five cases; between twelve and thirty-six hours in thirty-four cases; between thirty-six and forty-eight hours in eight cases; and later than this in only four cases. In many cases the first definite symptoms occurred suddenly, and evidently unexpectedly, but in some cases there were observed during the incubation more or less feeling of languor and ill health, loss of appetite, nausea, or fugitive, griping pains in the belly. In about a third of the cases the first definite symptom was a sense of chilliness, usually with rigors, of trembling, in one case accompanied by dyspnoea; in a few cases it was giddiness with faintness, sometimes accompanied by a cold sweat and tottering; in others, the first symptom was headache or pain somewhere in trunk of the body, *e. g.*, in the chest, back, between the shoulders, or in the abdomen, to which part the pain, wherever it might have commenced, subsequently extended. In one case the first symptom noticed was a difficulty in swallowing. In two cases it was intense thirst. But however the attack may have commenced, it was usually not long before pain in the abdomen, diarrhoea, and vomiting came on, diarrhoea being of more certain occurrence than vomiting. The pain in several cases commenced in the chest or between the shoulders, and extended first to the upper and then to the lower part of the abdomen. It was usually very severe indeed, quickly producing prostration or faintness, with cold sweats. It was variously described as crampy, burning, tearing, etc. The diarrhoeal discharges were in some cases quite unrestrainable, and (where a description of them could be obtained) were said to have been exceedingly offensive and usually of a dark color. Muscular weakness was an early and very remarkable symptom in nearly all the cases, and in many it was so great that the patient could only stand by holding on to something. Headache, sometimes severe, was a common and early symptom; and in most cases there was

thirst, often intense and most distressing. The tongue, when observed, was described usually as thickly coated with a brown, velvety fur, but red at the tip and edges. In the early stage the skin was often cold to the touch, but afterward fever set in, the temperature rising in some cases to 101°, 103°, and 104° F. In a few severe cases where the skin was actually cold, the patient complained of heat, insisted on throwing off the bedclothes, and was very restless. The pulse in the height of the illness became quick, counting in some cases 100 to 128. The above were the symptoms most frequently noted. Other symptoms occurred, however, some in a few cases, and some only in solitary cases. These I now proceed to enumerate: Excessive sweating, cramps in the legs, or in both legs and arms, convulsive flexion of the hands or fingers, muscular twitchings of the face, shoulders, or hands, aching pain in the shoulders, joints, extremities, a sense of stiffness of the joints, prickling or tingling or numbness of the hands lasting far into convalescence in some cases, a sense of general compression of the skin, drowsiness, hallucinations, imperfection of vision, and intolerance of light. In three cases (one that of a medical man) there was observed yellowness of the skin, either general or confined to the face and eyes. In one case, at a late stage of the illness, there was some pulmonary congestion, and an attack of what was regarded as gonit. In the fatal cases, death was preceded by collapse like that of cholera, coldness of the surface, pinched features, and blueness of the fingers and toes and around the sunken eyes. The debility of convalescence was in nearly all cases protracted to several weeks.

“The mildest cases were characterized usually by little remarkable beyond the following symptoms, viz.: abdominal pains, vomiting, diarrhoea, thirst, headache, and muscular weakness; any one or two of which might be absent.”

The cause of this illness was traced conclusively to the hams eaten. KLEIN found in the meat a bacillus, cultures of which were used for inoculating animals. These inocula-

tions were found generally to be followed by pneumonia. No attempt was made to isolate a ptomain.

Later, BALLARD reported fifteen cases with symptoms similar to the above, and with one death, from eating baked pork. Not all of those who ate of this pork were made sick. This might have been due to inequality in the putrefactive changes in different portions of the meat, or it may have been due to differences in temperature in various portions of the meat during the cooking. In the blood, pericardial fluid, and lungs of the fatal case, KLEIN observed bacilli similar to those discovered in the Wellbeck inquiry. Pneumonia was produced by inoculating guinea-pigs and mice with these bacilli.

More recently still, BALLARD has reported the following additional instances:

The Chester Case.—A man ate of some so-called American sausage, which consisted mostly of pork. Gastro-enteric symptoms with great prostration resulted, and in a few days the man died apparently from pneumonia. No postmortem examination was permitted, but the meat killed animals fed with it. In these were found hemorrhage in the stomach, congestion of the lungs, and hyperæmia of the medullary portion of the kidneys. "Most of the urinary tubules contained casts, while many of the Malpighian corpuscles, with their surrounding tissues, were in a state of disintegration, without, however, any inflammatory cells being present, indicating the disintegration to be due to the direct result of some destructive agency circulating in the vessels."

The Oldham Case.—Members of two families partook of a newly opened can of pigs' tongues. Nausea, vomiting, and diarrhœa occurred in all but one, and he fell into a comatose condition, which was not relieved until a purgative was administered and acted. This is suggestive of the fact that in the treatment of most cases of food-poisoning the vomiting and purging should be regarded as curative means, and when not present the danger is increased unless the removal of the

poison from the body be accomplished by inducing one or both of these processes.

The Bishop Stortford Case.—Members of three families ate of ribs of beef. The meat was cooked on Saturday, and it was more poisonous on Monday than on the preceding day. This meat evidently became infected after it was cooked. Other portions of the same carcass caused no ill effects.

The Whitechurch "Brawn" Case.—Brawn is a gelatin made from pig's head, and eaten cold. Members of ten families residing in different parts of the town were affected. The preparation was made September 7th. Only the members of one family of those who ate of it the next day were affected, while all of those who partook of it on the second day were made ill.

The Whitechurch Pork Case.—"The pork was provided for a Sunday family dinner, at which it was eaten freshly cooked and hot; it was eaten cold at supper, and cold again at dinner on Monday; it was eaten also cold by a man and his wife residing three miles distant, both of whom died from its effects after about thirty hours' illness. The others recovered. There appears to be evidence that the pork eaten, whether hot or cold, on Sunday did no mischief. It was not until Monday that it made people ill. There is this further interesting and instructive fact to be noted, viz., that those who ate the pork at dinner only on Monday were not attacked until after an interval of seven to nineteen or more hours, while the two persons who ate it in the evening and died were attacked much more quickly, namely, about four hours after eating."

The Wolverhampton Tinned Salmon Case.—Three adults ate, and two children merely tasted, some canned salmon. The can was "blown" and the contents partially decomposed. The one who ate the most was attacked about ten hours after eating, and died in three days. One who ate less became ill in about twelve hours, and died in five days; while the adult who partook most sparingly began to feel the ill effects in about fourteen hours, and finally recovered. In the children

the symptoms were slight and transient. KLEIN found in both fatal cases necrosis of the superficial layers of the mucous membrane of the stomach, fatty degeneration of the liver as in acute phosphorous poisoning, and inflammation of the kidneys. Mice fed upon the salmon died and exhibited lesions similar to those found in the men. No germ could be found in the blood of the mice.

The Curlisle (A) Case.—Twenty-four persons partook of a cold breakfast, which had been prepared the previous day and kept in a cellar in which milk and meat “were known to go bad.” Two persons died. The harmful article seems to have been “American ham,” although the jellies and game pie were also probably infected. The period of incubation was from six to forty-three hours. The only germ which could be found was a micrococcus, which was harmless, when fed by mouth, to mice, cats, and dogs.

The Iron Bridge Case.—Twelve persons out of fifteen in a household ate at mid-day of veal pies which had been made the day before and warmed over. “The unused pie was forwarded to KLEIN after it had been kept for thirteen days, as well as a portion of the raw veal from which it had been made. The raw veal produced no ill results on mice, a dog, and a cat fed with it, nor did the pie until the feeding had been continued for three days, when two of the mice died. Postmortem examination gave evidence of intestinal inflammation and congestion of the kidneys, but no organism was detected in the blood or viscera. This pie, when opened, was found to be mouldy, and there was a whitish scum which was an almost pure culture of a species of motile bacterium resembling the bacterium termo(?), and between the pieces of meat and in the jelly of the pie were two kinds of motile bacilli. All these organisms were cultivated. No ill effects followed feeding or inoculation with the cultivations of any of these organisms except one, the special kind of bacterium termo(?) just mentioned. This bacterium and its culture characteristics are fully described by Dr. KLEIN in the Report of the Medical Officer of the Board for 1890, p. 194.

One curious thing about it is that it does not grow well in any medium at a temperature above 30° or 32° ; at 36° to blood-heat no growth takes place. A very interesting fact about all its cultures is that after some days' growth the cultures possess a most exquisite and delicate aromatic odor, no trace of putridity being perceptible. Experiments made with the pure cultivation of this bacterium resulted as follows: As might be expected from the fact that it does not grow at blood-heat, subcutaneous inoculations into mice produced no results; but when mice were fed with the contents of a culture-tube, they fell ill and died, the customary lesions in the stomach and intestines, lungs, liver, and kidneys being found after death, but no bacterium was to be found in any of the viscera. The obvious inference from this is that the cultures of this bacterium contained a substance which, when introduced into the stomach, produced illness and death, in the latter event severe gastro-enteritis being a conspicuous feature. Since the organism in itself is harmless when inoculated, not being capable of growth and multiplication at the temperature of the animal body, it follows that the substance which effected the poisoning was non-organized and produced by the above bacterium termo."

The Retford Case.—Eighty persons in twenty-two families were made ill, one fatally, by eating pork pie. The pie was cooked on the 10th of November, and was eaten from the 11th to the 14th. With the exception of one family, none of those who ate of the pie on the 11th were made ill, and none of those who ate of it after the 14th. The harmful germ was a short bacillus, cultivations of which made mice sick, killing some; but cultures more than ten days old were without effect.

The Carlisle (B) Case.—A pork pie was made November 1st and eaten by some twenty-five persons during the following ten days. Mice fed with the meat developed on the second or third day a bloody diarrhoea and died. The small intestine was filled with bloody mucus. The lungs were congested, and in the animals which lived the longest there was

hepatization, chiefly in the upper lobes. The liver and spleen were also congested.

The Portsmouth Case.—Two bacilli—one motile, the other not—were found in the food. When first received, the meat poisoned mice fed upon it, but after standing and becoming offensive in odor it failed to do so. Cultures of the non-motile bacillus had a pleasant, aromatic odor, and were poisonous; while those of the motile germ were offensive and harmless. In the animals killed by the culture the lungs were found dark red and almost hepatized. The liver, kidneys, and spleen were also dark. The small intestines were relaxed and filled with mucus. In only one of the mice could the bacillus be found. KLEIN states that the bacillus was not pathogenic, but that its cultures contained a chemie poison.

The Middlesborough Pneumonia Epidemic.—This is so extraordinary in the history of food-poisoning that we shall quote BALLARD's summary of it in full: "This epidemic, which prevailed during the early part of 1888, and resulted in 490 deaths during the year in a population of about 98,000 persons, largely composed of iron-workers, was intrusted to me for investigation. The prevalent disease was proved incontestably to be of infectious character, communicable from person to person by proximity, through the medium of sewer air(?), and in other ways in which other infectious fevers are known to be spread. But all this left unexplained a curious geographical limitation of the epidemic which puzzled me greatly, until calling to mind some old experiences, I began to study the food and mode of life of the classes principally attacked. I then obtained the clue I wanted, in the double fact that during the greater part of every week nearly the only animal food these people got was what is termed "American bacon," made by soaking in water and then only partly drying salted pork imported from America; and that the limitation of the epidemic corresponded closely with a similar geographical limitation of the wholesale trade of a manufacturer of this bacon in the town of Middlesborough, who in the affected districts had by far the largest portion of this

trade in his hands. This was the thread which, by following it along, led me to the discovery I am about to mention. I sent to Dr. KLEIN, for examination and experiment, fresh portions of viscera (as well as hardened portions) of persons dead with the malady, and also specimens of the suspected bacon of which sick persons had recently eaten, and similar specimens purchased at different retail shops in the district. The result of Dr. KLEIN's work upon these materials was briefly this: That the disease I was dealing with was, as I suspected, no new local malady, but a specific, general disease or fever, marked, as other general, specific diseases are, by destructive morbid changes in all the principal viscera, the special characteristic of which, in this case, was a pleuropneumonia. In the lung juice and in the lung tissue Dr. KLEIN discovered a hitherto undescribed short bacillus, which he has called "*bacillus pneumoniae*," differing altogether from the bacillus of Friedländer and from the diplococcus pneumoniae of Fraenkel and Weichselbaum, neither of which was present. Of twenty samples of bacon forwarded from the infected districts, fourteen were distinctly poisonous to rodents fed with it; in two instances there was some doubt, and only four proved not to be poisonous. In the dead animals, lung lesions and lesions of other viscera similar to those observed in persons who died of the disease in the infected districts were found. Similar results followed inoculation of the human lung juice and of pure cultivation of the bacillus pneumoniae. In all instances the bacillus was recoverable. An instructive incident in Dr. KLEIN's part of the investigation was this, viz., that during its progress an epidemic of pneumonia occurred among the animals (mice, guinea-pigs, and monkeys) kept in the building where his experiments were carried on, the bacillus pneumoniae being found after death in the lung juice and sometimes in the heart's blood also. Another fact must be mentioned, namely, that on re-examination, after the lapse of three months, of samples of the bacon that had previously produced illness and death, they were found to have lost their powers of infecting ani-

mals, and no growth of the bacillus was obtainable. The details of the evidence on which I base this connection between the use of the suspected kind of bacon and the spread of the epidemic disease will be found in my detailed report on the epidemic in the Eighteenth Annual Report of the Medical Officer of the Local Government Board."

In meat which poisoned a large number of persons, GÄRTNER found his *bacillus enteritidis*. The meat was from a cow that had a severe diarrhœa for two days before she was killed. Of twelve persons who ate the flesh raw, all were sick; while of those who ate of the cooked food a large per cent. were also affected. In the meat and in the spleen of a person who died from the effects of the poison, GÄRTNER found the bacillus which proved fatal to animals. Good beef, inoculated with this bacillus and cooked some hours, killed rabbits, guinea-pigs, and mice. The skin of the people who were poisoned and recovered peeled off. The period of incubation varied from two to thirty hours. Even the boiled bouillon cultures of this germ are highly poisonous, showing that the toxic products are not destroyed by the cooking of the meat.

FISCHER reports the following: A cow, that had recently calved, had been sick for some eight days. As a result she was much reduced in weight and on account of the illness she was killed. The animal was slaughtered on Friday, and on the following Sunday at noon nineteen persons ate of the meat. The prominent symptoms were vomiting and violent purging, these symptoms appearing a few hours after the meal. Vertigo, loss of consciousness, and exfoliation of the epidermis during recovery, all of which were observed by GÄRTNER in some of his cases, were not present in any of FISCHER'S. Notwithstanding these differences, however, a study of the bacillus found in the meat led FISCHER to the conclusion that it is identical with the bacillus enteritidis of GÄRTNER. By evaporating a filtered culture and precipitating the concentrated fluid with absolute alcohol, the poison was obtained. It gave the general reactions for peptons, and boiling for one and one-

half hours did not perceptibly weaken its toxic properties, therefore cooking will not render meat infected with this germ harmless.

LUBARSCH has reported the death of a child two days old from a septic pneumonia caused by the bacillus enteritidis of GÄRTNER. About the close of the first twenty-four hours, the nurse noticed that the stools were of very disagreeable odor and greenish. However, the child rested well through the night. The next day the stools continued foul, the child became cyanotic, and the respirations increased to 60. Nothing could be detected in the lungs by either auscultation or percussion. The breathing was wholly costal, and the abdomen somewhat distended. There was no evidence of infection through the umbilicus. The urine contained no albumin and no hemoglobin. The diagnosis was Winkel's disease.

The anatomical examination showed pleuritis and pneumonia of the left lower lobe, bilateral purulent bronchitis, atelectasis of the right lung, parenchymatous cloudiness of the kidneys, fatty infiltration and engorgement of the liver, slightly enlarged spleen, uric acid infarction of the kidneys, and icterus neonatorum. All other pathological conditions were supposed to be consequent upon the septic pneumonia. Agar-agar plates were made from the lower lobe of the left lung and the spleen. These developed only one germ. The superficial colonies were grayish-white, the size of pin-heads, and slimy. The deep ones were white and only half as large as the superficial ones. The bacilli were mostly small and oval, with a few longer ones. In hanging drops they showed a decided but not very active motility. Coloring with a one per cent. aqueous solution of gentian-violet or with a two per cent. one of methylen-blue was not very intense. On the other hand, with Loeffler's potash or Sahli's borax-methylen-blue solution, the coloration was excellent. On gelatin plates the growth was practically the same as on agar-agar. However, the colonies were whiter and retained this color for a longer time. Some of the bacilli showed a deeper stain in the middle, when colored, than at the poles. However, this was not frequently the case. In stich cultures,

the bacillus grows slowly along the line, forming a grayish-white cap and failing to liquefy. Streak cultures on agar-agar were grayish-white or grayish-yellow, and slightly transparent along the borders. It clouded bouillon and formed a yellowish sediment. On potatoes, the growth was plainly visible after twenty-four hours at 37° and formed a grayish-white or grayish-yellow layer. White rats and chickens proved to be wholly immune, while guinea-pigs, rabbits, and white mice were susceptible. The susceptible animals were killed within from sixteen to twenty-four hours by intraperitoneal inoculation and in from two to four days by subcutaneous injections. In all cases, section showed marked congestion of the intestines, swelling of the follicles, and in some instances slight hemorrhages in the mucous membrane. After intraperitoneal inoculation, sero-fibrinous or hemorrhagic peritonitis developed. After subcutaneous inoculation in rabbits, sometimes in guinea-pigs, there was a sero-fibrinous pleuritis with compression of the lungs, and in one instance a circumscribed pneumonia. Sterilized cultures in larger quantities produced the same effects as the unsterilized. GÄRTNER, to whom specimens of this bacillus were sent, regards it as a variety of the bacillus enteritidis.

The symptoms and anatomical changes produced by this bacillus agree with those observed in Winkel's disease in the rapidly fatal progress, cyanosis, icterus, rapid respiration, tending to hemorrhage and fatty degeneration. The most essential difference lies in the fact that hemoglobinuria is a prominent symptom in Winkel's disease.

LUBARSCH discusses the probable avenues through which the infection may have occurred in this case. He admits that RUNGE is probably right in the claim that infection of the newly born may occur through the cord without leaving any recognizable changes. If this was the method of infection in this case, the germ must have been carried to the lower lobe of the left lung by the blood. Why to this part of the body rather than to another cannot be determined. The bacillus may have found its way into the mouth of the child from the genitals of the mother, who developed a parametritis after

delivery, but the germ could not be found in the secretion of these parts. Lastly, the germ may have been inhaled.

August 29, 1887, 256 soldiers and 36 citizens at Middleburg, Holland, were taken sick after eating meat from a cow which had been killed while suffering from puerperal fever. The symptoms were nausea, vomiting, purging, elevation of temperature, and prostration. In some there were observed dizziness, sleepiness, and dilatation of the pupil. After a few days these symptoms gradually disappeared, and in many, an eczematous eruption of the lips gave annoyance. Pigs, cats, and dogs which ate of the offal of this animal were also made sick. Thorough cooking did not destroy the poison, and those who took soup and bouillon made from the meat were affected like those who ate of the muscular fibre. In most of the cases the symptoms came on within twelve hours after eating the meat.

BASENAU reports the following: On the 15th of March, 1893, a cow, that had calved eight days before, was brought to the Amsterdam abattoir. At that time the animal was very sick, and in consequence of this condition she was killed four days later. It was impossible to get any exact information concerning the sickness, but in all probability it was puerperal fever. On account of the changes in the viscera due to the disease, the director of the abattoir forbade the sale or consumption of the flesh. About three kilos from the gluteal region was sent to the hygienic institute for examination. The meat was pale red, feebly acid, normal in consistency, and of slightly stale odor. A broad, heated knife was drawn over the surface repeatedly in order to destroy germs that may have found lodgement there. With a second sterilized knife a deep vertical incision. With a third and a fourth sterilized knife horizontal sections were made. From the interior of the piece thus laid bare the material for cultures was taken.

One portion was rubbed up in a sterilized mortar, and a bit the size of a pea was placed under the skin at the root of a mouse's tail. A second mouse was fed with about two grams of the meat rubbed up with bread.

Streak preparations from the meat stained with Loeffler's methylen-blue showed short rods, two to two and one-half times long as broad, mostly in pairs, sometimes single, and at other times in groups.

The gelatin plates developed after twenty-four hours. To the eye, the colonies appeared of pin-head size and of yellow color, the intensity of which increased with age. The surface colonies showed brown centres with broad glistening edges. By transmitted light the yellow centre became lighter and the outer zone greenish-blue, which color did not extend into the gelatin beyond the colony.

Under a low power, the colonies appeared yellow with a dark contour. The yellow portion was filled with light and dark stars.

From the number of colonies that developed, it was estimated that one gram of the meat contained 187,500 bacilli.

The mouse which had the subcutaneous inoculation died after thirty, and the other one after thirty-six hours. Some eighteen hours after infection both animals became apathetic; with roughened coats and closed eyes they remained on their sides.

Section of the mouse inoculated subcutaneously showed the following: The place of inoculation had become a yellowish-white, broken down mass. The surface of the liver was covered with pin-head, grayish-white foci of infection, which gave the organ a marbled appearance. The spleen showed a similar infection, and both organs were somewhat enlarged. No changes were observed microscopically in other organs.

The mouse infected by feeding showed in addition to the changes observed in the other one marked injection of the intestinal bloodvessels and a rose-red coloration of the intestinal mucous membrane.

The following is a condensed description of this germ to which BASENAU has given the name *bacillus bovis morbi-cans*:

Small rods from 1 to 1.2 mm. long and 0.3 to 0.4 mm. broad.

It clouds bouillon within twenty-four hours at 37°, forming

a smooth serum on the surface, which easily breaks on being shaken, and sinks. In old cultures a grayish-white deposit forms, while the fluid remains cloudy. In stich gelatin cultures, it forms along the line a small, yellowish-white band, smooth along the centre and freely feathered along the edges. It slowly forms a round white cap on the surface. It does not liquefy.

On streak gelatin cultures this germ grows abundantly, forming a thick layer which by transmitted light shows a greenish-blue tint. This growth resembles those of the coli commune and the typhoid bacillus.

The gelatin colonies have already been described.

On agar-agar there is found within twenty-four hours at 37° an abundant growth, grayish-white, and extending over the entire surface.

On potatoes the growth is less rapid, yellow, never changing to brown.

On blood serum it forms a broad, thin, glistening layer, with a cloudiness of the water of condensation.

In milk it grows rapidly without coagulating the casein.

It is a facultative anaërobe and does not produce gas, except in bouillon, containing one per cent. of grape sugar, and even in this the amount evolved is small.

It is motile and stains well with all the basic anilin dyes, best with fuchsin and methylen-blue. It is decolorized by Gram's method, although slowly.

This bacillus proved to be pathogenic to mice, rats, guinea-pigs, rabbits, and calves. A bouillon culture was injected into the uterus of a guinea-pig a few hours after she had given birth to three young. Death of the mother and of the young, from taking of the infected milk, resulted. The germ was found in the mammary glands, and, in fact, in the blood, milk, liver, spleen, peritoneal fluid, and muscles.

In a second communication, BASENAU shows that the bacillus is found in the milk of animals inoculated with it, and that newly drawn, sterile milk has no germicidal effect upon it.

December 31, 1894, Dr. TRAYER, of Somerset, Mich.,

was called to see the family of Mr. Van Allen. He found the father, mother, and two children suffering from protracted vomiting and marked exhaustion. There was no fever and no diarrhœa. Tyrotoxicon poisoning was suspected, but inquiry showed that their supper had consisted of bread, butter, tea, dried beef and raspberry sauce. The dried beef was examined by VAUGHAN and PERKINS. There was nothing in the appearance or odor of the meat to cause any suspicion. In fact, it seemed to be of exceptionally good quality. Anaerobic cultures from the interior of the meat were made and developed a bacillus, from two to three times as long as broad, taking the ordinary stains well, motile, with no spore formation, not liquefying gelatin, but coagulating milk, growing best at the temperature of the body, but developing its poison at ordinary temperature, producing gas abundantly, and pathogenic to white rats, rabbits, and guinea-pigs. Sterilized cultures were also poisonous.

Of 200 men at a banquet at Sturgis, Mich., April 26, 1894, every one who ate of the pressed chicken served was made sick. Some men who were not at the banquet, but who aided in preparing for it, took small bits of the chicken, and these were also sick. All were taken within from two to four hours after eating the chicken with nausea, violent griping and purging; many fainted while attempting to arise from bed.

The chickens were killed Tuesday afternoon, picked and left hanging in the market-room (not in cooling-room) until Wednesday forenoon, when they were drawn and carried to a restaurant, and here left in a warm room until Thursday morning, when they were cooked (not very thoroughly), pressed and served at the banquet that night. Those who ate of the compressed chicken were also made sick.

The pressed chicken was examined by VAUGHAN and PERKINS. It contained two microorganisms, a slender bacillus from four to five times as long as broad, and a streptococcus. The bacillus was fatal to white rats, guinea-pigs and rabbits, when administered intraperitoneally, intravenously, and sub-

cutaneously. The streptococcus was not fatal when given in pure culture, but mixed cultures of the two induced death; and in these instances, when administered subcutaneously, in addition to lesions found after the employment of pure cultures of the bacillus, there was extensive sloughing. This bacillus is motile, takes the ordinary stains readily, and is decolorized by Gram's method. It grows very slowly at ordinary temperature, and rapidly at 37° . Of two cultures of equal age, one grown at ordinary temperature and the other at 37° , one c.c. of the former was necessary to induce death, while one fourth c.c. of the other proved fatal. The anaerobic cultures were much more powerful than the aerobic. On gelatin plates exposed to the air the growth was slow, while on those kept in an atmosphere of hydrogen it was much more rapid. Spores could not be detected. The bacillus coagulates milk and decolorizes litmus gelatin. On potatoes it forms a dirty, thick, slimy growth. It does not liquefy gelatin, and the production of gas was not observed. Streaks on agar-agar are yellowish-white, slimy, and with but little tendency to spread. One-half c.c. of a beef-tea culture heated to 60° for thirty minutes killed. One c.c. heated to 100° for fifteen minutes failed to kill. Animals inoculated by the methods mentioned showed evidences of abdominal pain within from one to two hours, and several were found dead after twelve hours. The abdominal cavity was found filled with a clear fluid, the bloodvessels were much congested, and the peritoneum reddened. In some instances a bloody fluid was found in the pleural cavity.

In an outbreak in an asylum for the insane at Gaustad in Norway the patients' food was veal. HOLST found that the effects were due to a small bacillus not identical with that of Gärtner.

LEWIS found a ptomain, which he supposed to be neuridin, in corn-beef that poisoned people in Ohio.

POELS reports an interesting instance of meat-poisoning in Rotterdam. The cow that furnished the flesh was supposed to be healthy. The flesh contained a short bacillus, believed by POELS to be a variety of the bacillus coli, which was patho-

genic to mice, rabbits, and calves. The intravenous injection of a pure culture of this bacillus caused death in a calf from a profuse bloody diarrhœa within five hours. The blood was dark-red and not coagulated; the kidneys and liver were engorged; all the lymph glands were swollen; the mucous membrane of the intestines showed numerous hemorrhagic spots, and the bacilli were found in the blood, all the organs, and in the muscles. Even filtered cultures killed calves. This demonstrated the marked toxicity of the chemie products.

In another outbreak of meat-poisoning in a distant part of Holland, POELS found the same bacillus in the flesh.

ZORKENDORFER reports the presence of anthrax bacilli in some meat that poisoned many people, some fatally, near Tep-litz, in 1894. However, his identification of the germ cannot be regarded as positive. Anthrax was not known to be present at the time among the animals in that neighborhood. One of the hogs that furnished a portion of the meat was known to be sick when slaughtered, but the nature of the disease was not known. This, together with the fact that the bacillus found in the meat and in the tissues of the fatal cases did not correspond with the typical anthrax bacillus, renders the conclusion drawn by this investigator of doubtful accuracy.

DI MATTEI states that the flesh of animals dead from symptomatic anthrax may retain its power of infection after having been preserved in a dry state for ten years.

On a fête-day at Zurich, in 1839, 600 persons who were fed upon cold veal and ham were taken ill, with shivering, giddiness, vomiting, and diarrhœa. Some were delirious and others were salivated, the saliva being extremely fetid. In the worst cases there were involuntary stools, collapse, and death. The cause was traced to putrefactive changes in the meat.

SIEDLER reports an instance of four persons having been made sick by eating decomposed goose-grease. There were giddiness, prostration, and violent vomiting. No metallic poison could be found. The grease was rancid, of repulsive odor, and three ounces of it given to a dog produced the same symptoms which had been observed in the persons.

CHRISTISON reports a number of cases in which persons were seriously, a few fatally, affected by eating various kinds of meat which had undergone partial putrefaction.

OLLIVIER found six persons poisoned, four of them fatally, by eating of decomposed mutton. He also mentions the poisoning of a family of three with ham pie. Chemic analysis ailed to reveal the presence of any poison.

BOUTIGNY, having failed to find any poison in the meat furnished at a festival, and to which the serious illness of many was attributed, made a meal of stuffed turkey furnished by the same dealer, but after a short time his countenance became livid, his pulse small and feeble, a cold sweat bathed his body, and violent vomiting and purging followed. His recovery was slow.

GEISELER observed nausea, vomiting, purging, and delirium after eating of bacon which was imperfectly cured.

Cases of poisoning from eating canned meats have become quite frequent. Although it may be possible that in some instances the untoward effects result from metallic poisoning, in the great majority of cases the poisonous principles are formed by putrefactive changes. In many instances it is probable that decomposition begins after the can is opened by the consumer. In others, the canning is carelessly done and putrefaction is far advanced before the food reaches the consumer. In still other instances, the meat may be taken from diseased animals, or it may undergo putrefactive changes before the canning. What is true of canned meats is also true of canned fruits and vegetables.

DR. ASHWORTH, of Smithland, Iowa, has reported to us three fatal cases of poisoning from canned apricots. An infant which was only eight days old, and which must have received the poison from its mother's breasts, died within a few hours. The mother died forty-three hours after eating the apricots, and the father on the sixth day. The symptoms corresponded with those of poisoning by tyrotoxon. However, it seems that no analysis was made, and these may have been cases of mineral poisoning.

POISONOUS CHEESE.—In 1827 HÜNNEFELD made some analyses of poisonous cheese, and experimented with extracts upon the lower animals. He accepted the ideas of KERNER in regard to poisonous sausage in a somewhat modified form, and thought the active agents to be sebacic and caseic acids. About the same time, SERTÜRNER, making analyses of poisonous cheese for WESTRUMB, also traced the poisonous principles, as he supposed, to these fatty acids. We see from this that during the first part of the present century the fatty acid theory, as it may be called, was generally accepted.

In 1848, CHRISTISON, after referring to the work of HÜNNEFELD and SERTÜRNER, made the following statement: “His (Hünnefeld’s) experiments, however, are not quite conclusive of the fact that these fatty acids are really the poisonous principles, as he has not extended his experimental researches to the caseic and sebacic acids prepared in the ordinary way. His views will probably be altered and simplified if future experiments should confirm the late inquiries of Braconnot, who has stated that Proust’s caseic acid is a modification of acetic acid combined with an acrid oil.”

In 1852 SCHLOSSBERGER made experiments with the pure fatty acids and demonstrated their freedom from poisonous properties. These experiments have been verified repeatedly, so that now it is well known that all the fatty acids obtainable from cheese are devoid of poisonous properties.

It may be remarked here that there is every probability that the poisonous substance was present in the extracts obtained by the older chemists. Indeed, we may say that this is a certainty, since the administration of these extracts to cats was, in some instances at least, followed by fatal result. The great mass of these extracts consisted of fatty acids, and, as the chemists could find nothing else present, they very naturally concluded that the fatty acids themselves constituted the poisonous substance.

Since the overthrow of the fatty acid theory various conjectures have been made, but none worthy of consideration.

We make the following quotations from some of the best

authorities who wrote during the first half of the past decade upon this subject :

HILLER says : " Nothing definite is known of the nature of cheese poison. Its solubility seems established from an observation by Husemann, a case in which the poison was transmitted from a nursing mother to her child."

HUSEMANN wrote as follows : " The older investigations of the chemic nature of cheese poison, which led to the belief of putrefactive cheese acids, and other problematic substances, are void of all trustworthiness, and the discovery of the active principle of poisonous cheese may not be looked for in the near future, on account of the proper animals for controlling the experiments with the extracts, as dogs can eat large quantities of poisonous cheese without its producing any effect."

BRIEGER stated in 1885 : " All kinds of conjectures concerning the nature of this poison have been formed, but all are even devoid of historical interest ; because they are not based upon experimental investigations. My own experiments toward solving this question have not progressed very far."

In the above quotation we think that BRIEGER has hardly done justice to the work of HÜNNEFELD and SERTÜRNER. Their labors can hardly be said to be wholly devoid of historical interest, and they certainly did employ the experimental method of inquiry.

In the years 1883 and 1884 there were reported to the Michigan State Board of Health about three hundred cases of cheese poisoning. As a rule, the first symptoms appeared within from two to four hours after eating the cheese. In a few the symptoms were delayed from eight to ten hours and were very slight. The attending physicians reported that the gravity of the symptoms varied with the amount of cheese eaten, but no one who ate of the poisonous cheese wholly escaped. One physician reported the following symptoms : " Everyone who ate of the cheese was taken with vomiting, at first of a thin, watery, later a more consistent reddish-colored substance. At the same time the patient suffered from diarrhœa with watery

stools. Some complained of pain in the region of the stomach. At first the tongue was white, but later it became red and dry; the pulse was feeble and irregular; countenance pale, with marked cyanosis. One small boy, whose condition seemed very critical, was covered all over the body with bluish spots."

Dryness and constriction of the throat were complained of by all. In a few cases the vomiting and diarrhoea were followed by marked nervous prostration, and in some dilatation of the pupils was observed.

Notwithstanding the severity of the symptoms in many, there was no fatal termination among these cases, though several deaths from cheese poisoning in other outbreaks have occurred. Many of the physicians at first diagnosed the cases from the symptoms as due to arsenical poisoning, and on this supposition some administered ferrie hydrate. Others gave alcohol and other stimulants and treated upon the expectant plan.

VAUGHAN, to whom the cheese was sent for analysis, made the following report: "All of these three hundred cases were caused by eating of twelve different cheeses. Of these, nine were made at one factory, and one each at three other factories. Of each of the twelve I received smaller or larger pieces. Of each of ten I received only small amounts. Of each of the other two I received about eighteen kilograms. The cheese was in good condition and there was nothing in the taste or odor to excite suspicion. However, from a freshly cut surface there exuded numerous drops of a slightly opalescent fluid which reddened litmus paper instantly and intensely. Although, as I have stated, I could discern nothing peculiar in the odor, if two samples, one of good, the other of poisonous cheese, were placed before a dog or cat, the animal would invariably select the good cheese; but if only poisonous cheese was offered, and the animal was hungry, it would partake freely. A cat was kept seven days and furnished only poisonous cheese and water. It ate freely of the cheese and manifested no untoward symptoms. After

the seven days the animal was etherized and abdominal section was made. Nothing abnormal could be found. I predicted, however, in one of my first articles on poisonous cheese, that the isolated poison would affect the lower animals. As to the truth of this prediction we will see later.

"My friend, Dr. Sternberg, the eminent bacteriologist, found in the opalescent drops above referred to numerous micrococci. But inoculations of rabbits with these failed to produce any results.

"At first I made an alcoholic extract of the cheese. After the alcohol was evaporated in vacuo at a low temperature a residue consisting mainly of fatty acids remained. I ate a small bit of this residue, and found that it produced dryness of the throat, nausea, vomiting, and diarrhoea. The mass of this extract consisted of fats and fatty acids, and for some weeks I endeavored to extract the poison from these fats, but all attempts were unsuccessful. I then made an aqueous extract of the cheese, filtered this, and, drinking some of it, found that it also was poisonous. But after evaporating the aqueous extract to dryness on the water-bath at 100°, the residue thus obtained was not poisonous. From this I ascertained that the poison was decomposed or volatilized at or below the boiling-point of water. I then tried distillation at a low temperature, but by this the poison seemed to be decomposed.

"Finally, I made the clear, filtered aqueous extract, which was highly acid, alkaline with sodium hydrate, agitated this with ether, removed the ether, and allowed it to evaporate spontaneously. The residue was highly poisonous. By resolution in water and extraction with ether, the poison was separated from foreign substances. As the ether took up some water, this residue consisted of an aqueous solution of the poison. After this was allowed to stand for some hours in vacuo over sulphuric acid, the poison separated in needle-shaped crystals. From some samples the poison crystallized from the first evaporation of the ether, and without standing in vacuo. This happened only when the cheese contained a

comparatively large amount of the poison. Ordinarily, the microscope was necessary to detect the crystalline shape. From sixteen kilograms of one cheese, I obtained about 0.5 gram of the poison, and in this case the individual crystals were plainly visible to the unaided eye. From the same amount of another cheese I obtained only about 0.1 gram, and the crystals in this case were not so large. I have no idea, however, that by the method used all the poison was separated from the cheese."

To this ptomain VAUGHAN has given the name tyrotoxon (τυρος, cheese, and τοξικον, poison). Its chemistry will be discussed in a subsequent chapter. The word tyrotoxon had been previously employed to designate poisonous cheese, but this was not known to the writer when the above work was done. In the *Universal Lexikon der praktischen Medicin und Chirurgie*, published at Leipzig in 1849, an interesting article under the heading Tyrotoxon may be found, and of which the following is a condensed translation :

"Tyrotoxon (from tyros, cheese, and toxicon, poison) cheese poison. With this name we designate the poisonous principle which is formed in cheese by putrefaction. Already frequent instances have occurred in which after the use of old cheese poisonous effects have followed, and in most recent times a few examples of this have been observed. Those which we here mention are from the work by Kuhn, from the English and Latin, Leipzig, 1824 (*Versuche und Beobachtungen über die Keesäure, das Wurst und Käsegift*).

"At first (we will give) a few words about the preparation of the cheese. When milk is allowed to stand for some days in broad, shallow bowls, freely exposed to the air, the cream separates and forms on the surface a layer, the thickness of which varies with the quality of the milk. If the cream be repeatedly removed, there remains a large volume of whey in which appears a silver-white, cheesy substance which is too soft to be made into cheese. This is stored in copper or earthen vessels until a considerable amount has been obtained. Then the vessel is heated until the cheesy material

becomes more firm. The substance is then placed in a linen bag and pressed between stones in order to remove the whey as thoroughly as possible. When no more fluid can be expressed, the solid mass is kneaded with salt and caraway, formed into a cheese, and dried in the open air. However, in different localities the method of preparation varies. It is now desirable to inquire into the chemical constitution of the constituents of the cheese. Thenard has written as follows concerning the casein, cheese-oxid, and cheese acid: One obtains casein by skimming coagulated milk, washing the coagulum with much water, and then drying. The mass is white, tasteless, and odorless, and changes neither the tincture of litmus nor the syrup of violets. On being burned, it evolves much ammonium carbonate and leaves a light coal, which is reduced to ashes with difficulty. In the ash there is much carbonate of lime. When combined with much water casein ferments as easily as gelatin, and in cheese it is converted into ammonium acetate, cheese-oxid, gum, etc. The properties of this cheese-oxid are lightness, capability of foaming, white color, lack of odor and taste, and inability to act upon the vegetable colors. In boiling alcohol it is only slightly soluble, in ether wholly insoluble. Finally, the cheese acid, which Thenard also describes, possesses a slightly yellow color, an acid, bitter, and at the same time cheesy taste, and is easily soluble in both water and alcohol. Chlorin does not cloud an aqueous solution of it, but an infusion of nut-galls does. Nitric acid converts it into oxalic acid, and in addition thereto, as Proust has shown, a small quantity of benzoic acid and a large amount of a yellow, bitter substance is formed. By combination with ammonia it forms a salt, which does not crystallize. This has a salty, bitter, slightly cheesy taste, and it possesses the peculiar property that even when fully neutralized it reddens, after twenty-four hours, blue vegetable colors.

The poisonous properties of cheese may be derived from (1) the milk used, (2) from admixture with poisonous substances in its preparation, and (3) from a peculiar putrefaction.

“(1) Milk may possess most dangerous properties when it comes from sick animals or from those which have eaten plants poisonous to man. Remer believes it improbable, but Lorry states that the milk of cows, with whose food *gratiola* (*g. officinalis*?) has been mixed, also colostrum (from any cow?), may be so poisonous that cheese prepared from these may cause death. Tozzetti makes a similar statement on the authority of Gmelin. He states that cheese prepared from the milk of goats which had eaten *Euphorbia cyparissias*, L., produced poisonous effects, and that in Rome like effects had followed the eating of cheese made from the milk of goats which had fed upon similar plants. This cheese had a very sharp taste. It also contained a yellow fluid in which the poisonous substance existed. Gmelin states on the authority of trustworthy writers that the milk of goats which have eaten of *Euphorbia helioscopia* and *E. edulis* is poisonous. It is stated in Philadelphia that a child who took the breast of a mother recently bitten by a rattlesnake died from the effects of the poison. More sad still is the history of a peasant whose cow had been bitten by a mad dog; because not only he, but his wife and four children, a maid, and a neighbor, and her four children, who had partaken daily of the milk of the bitten cow, died of the fearful disease, hydrophobia. On account of results of this kind, the authorities should forbid the sale of milk, butter, and cheese from diseased animals.

“(2) The injurious effects of cheese are sometimes due to harmful substances accidentally mixed with it in its preparation. In the following example the ill effects were due to the presence of a mould (*Mucor mucedo*, L.). Of seven men who partook of a cheese, which was mouldy both inside and outside, at noon, all became ill during the evening. They suffered from frequent vomiting and severe purging, followed by such prostration that they could hardly move their limbs. The cheese, being recognized as the source of the trouble, was thrown away. However, six servants found and ate it. A few hours later they also began to vomit and purge, and four of them, who had partaken most freely, were compelled to lie

in bed an entire day, and even on the following day they presented the appearance of persons recovering from a severe illness. Microscopic examination of the mould from the interior showed the brown spore-capsules observed in the mould on the outside of the cheese absent and many spherical spores of bluish color present.

The following is another example in which the poisonous properties of the cheese were due to accidental contamination: The curd was allowed to stand for some time in an unfinished beech tray. Fermentation took place, and the cheese prepared from the curd contained the bitter extract of the beech, was pale-red in color, and the smallest bit of it induced vomiting and long-continued diarrhœa. Similar, but poisonous seeds, have been mistaken for caraway (*Cuminum cyminum*, L.) and incorporated in the cheese. Hager mentions a case of this kind in which twenty-four persons were poisoned. The seeds used in this cheese were *hyoscyamus*. Garre mentions a similar case in which a peasant and his family partook of the cheese and cooked plums. These people became temporarily insane and ran about the streets, unconscious of what they did. A surgeon found them lying in a room in a state of apparent intoxication. In all, the tongue was red and thick, the eyes sunken and wild, and the pulse thready and rapid. The father, who had undressed and was lying on the bed, attempted to arise. He seemed dazed, laughed at the questions asked, and made irrelevant remarks. His tongue and eyes were inflamed. His wife plucked at her clothing, and was also irrational. The fourteen year old son, who evidently had taken the largest amount of the poison, reeled as if intoxicated, and attempted to bite the visitors. His eyes were wild, his face flushed, and his tongue highly inflamed. The sister was more rational. A servant was wildly insane. A visitor, who had also partaken of the cheese, remained in a nervous and excitable state for five days. All complained of weakness, heaviness of the limbs, dryness of the tongue, pressure on the eyes and dimness of vision. In another instance

cited by Orfila, white arsenic had been mixed with the cheese. . . .¹

"(3) Finally, it has been demonstrated by the most trustworthy observations that cheese in and of itself, without any additions, may become so powerfully poisonous that life may be endangered by its use. Of the well-known kinds of cheese, two are especially liable to become poisonous. These are cottage (schmier) and sour (barsch) cheese. The former is free from injurious properties when fresh, but as it grows old and ferments it may become highly poisonous. Pyl reports an instance in which a butcher, of Tübingen, and his three children ate at most one-fourth of a pound of cottage cheese, which produced within one hour severe abdominal pain, vomiting, and purging. A girl, four years old, had convulsions which terminated in death on the second day. The remainder of this cheese was examined, but no trace of metallic poison could be found. Sour cheese is made from buttermilk. The coagulum obtained by boiling this milk is mixed with caraway, seasoned with pepper and made into cheese. Hennemann reports thirty persons poisoned with sour cheese, one fatally. Vomiting, diarrhoea, and exhaustion were the chief symptoms. The gravity of the effects seemed to be in direct proportion to the quantity of cheese eaten. Another instance of poisoning with sour cheese is reported by Willich, who states that a severe wound on the leg, due to a fall while under the effects of the poison, did not bleed until the patient vomited freely."

During 1887, WALLACE found tyrotoxicon in two samples of cheese which had caused serious illness. The first of these came from Jeunesville, Pa., and the symptoms as reported to WALLACE by DOOLITTLE, who had charge of the cases, were as follows: "There were at least fifty persons poisoned by this cheese. There were also eight others who ate of the cheese, but felt no unpleasant effects; whether this was due

¹ This part of the article is devoted to the detection of copper and lead in cheese, and is devoid of interest, since the methods described are now antiquated.

to personal idiosyncrasy, or to an uneven distribution of the poison throughout the cheese, I am unable to say.

"The majority, however, comprising fifty or sixty persons, were seized, in from two to four hours after eating the cheese, with vertigo, nausea, vomiting, and severe rigors, though varying in their order of appearance and in severity in different cases. The vomiting and chills were the most constant and severe symptoms in all the cases, and were soon followed by severe pain in the epigastric region, cramps in the feet and lower limbs, purging and griping pain in the bowels, a sensation of numbness or pins and needles, especially in the limbs, and lastly, very marked prostration, amounting almost to collapse in a few cases.

"The vomit at first consisted of the contents of the stomach, and had a strong odor of cheese; afterward it consisted of mucus, bile, and in three or four of the severer cases blood was mixed with the mucus in small quantities. Microscopic examination of the same was not made, but to the eye it appeared as such. The vomiting and diarrhoea lasted from two to twelve hours; the rigors and muscular cramps, one to two hours. The diarrhoeal discharges, at first fecal, became later watery and light colored. No deaths occurred, and for the most part the effects were transient, and all that remained on the following day were the prostration and numbness; the latter occurred in about one-half the cases, and disappeared in from one to three days.

"Children, as a rule, seemed to suffer less than adults, and, of course, it was not possible to elicit as definite symptoms from them. The suddenness of the attack was remarked by all, some feeling perfectly well until the moment of attack. Nor did the symptoms seem to be in proportion to the amount of cheese taken; some of the severest cases declared they had not eaten more than a cubic inch of it. One of the severest cases was about six and one-half months pregnant, but no interference with pregnancy occurred. All the cheese which caused the sickness came from the same piece."

The second sample of cheese examined by WALLACE came

from Riverton, N. J. This outbreak included a smaller number of persons, all of whom recovered.

WOLFF has detected tyrotoxicon in cheese which poisoned several persons at Shamokin, Pa. The pores of this cheese were found filled with a grayish-green fungoid growth, though it is not supposed that this fungus was connected in any way with the poisonous nature of the cheese. Tests were made for mineral poison with negative results, after which tyrotoxicon was recognized both by chemie and physiologic tests. "A few drops of the liquid (extract), placed on the tongue of a young kitten, produced prompt emesis and numerous watery dejections with evident depression and malaise of the animal. A larger cat was similarly affected by it, though the depression and malaise were not so marked nor so long continued."

Cheese poisoning caused the death of several children in the neighborhood of Heiligenstadt in 1879, and there were many fatal cases from the same cause in Pymont in 1878. Unfortunately we have not been able to find any detailed account of either the symptoms or the postmortem appearances in these cases.

ENRIHART has published the history of some cases of poisoning from cheese, of which the following is an abstract: The family of a workman, consisting of eight persons, ate for supper 600 grams (about eighteen ounces) of Limburger cheese. The rind was covered with a heavy mould, while the interior had become fluid from putrefaction, and was of bitter taste. Three ate only of the mouldy rind, and these remained well. The next morning, the five who had eaten of the inner portion suffered from vertigo, nausea, vomiting, and abdominal pains; no stool. The father had convulsive movements of all the extremities. The pupils were dilated, and did not respond to light; there were double vision, cold sweat, skin cyanotic, abdomen distended, difficulty in swallowing, delirium, mild trismus, and temperature 40° . The temperature of the mother, on account of the great collapse, was subnormal. She had no convulsive movements, but there was prolonged

loss of consciousness. The pulse was small and thready, and threatened paralysis of the heart. Recovery was very slow. The others suffered only from gastro-enteric symptoms. EHRLHARDT discusses the question as to whether these symptoms were due to tyrotoxicon or to infection with microorganisms; but as we have not had access to his original paper, we do not know what his conclusions are. However, there cannot be much doubt that in those cases in which the organism is taken into the alimentary canal, it continues the elaboration of its poisonous products.

DOKKUM treated a cheese which had poisoned a number of persons in the following manner: One-half kilo was made into a paste and heated on the water-bath for two hours with two per cent. of hydrochloric acid, and filtered. The filtrate was evaporated on the water-bath to a syrup, keeping it acid, and then treated with absolute alcohol. The alcohol was evaporated, then another portion added, and this again expelled. The remaining fluid is made alkaline and now extracted with ether. The ether on evaporation leaves a crystalline base, which is insoluble in both hot and cold alcohol. It readily reduces ferric chlorid and potassium ferricyanid and liberates iodine from hydriodic acid. The amount of the hydrochlorate of this base obtained was 0.02 gram. Five milligrams injected into a frog caused paralysis and death in thirty minutes. DOKKUM thinks that this base is not tyrotoxicon, but a currare-like poison for which he suggests the name tyrotoxin.

In 1890 VAUGHAN made the following additional report on poisonous cheese:

"During the past two or three years we have received at the Hygienic Laboratory of Michigan University a number of samples of cheese which, it was claimed, had caused nausea and vomiting in those eating of them, and in which we were unable to detect tyrotoxicon. Some of these samples produced vomiting and purging in cats and dogs to which the cheese was fed directly. The evidence that these samples had been the actual cause of the sickness among the people

who had eaten of them was thus confirmed by the experiments upon the animals; but inasmuch as we were unable to detect the poison we were compelled to report as follows:

“‘The poisonous character of the cheese has been proven by experiments upon animals, but we have failed to demonstrate the nature of the poison. Tyrotoxicon could not be detected.’

“One sample of this class was found by Novy to be very poisonous. Some of this cheese was covered with absolute alcohol, and after standing in a dish for some weeks the alcohol was allowed to evaporate, then 100 grams of the cheese was fed to a young dog and caused its death within a few hours. Sterilized milk to which a small bit of the cheese was added, after standing in the incubator at 35° for twenty-four hours, became so poisonous that 100 c.c. of it introduced into the stomach of a full-grown cat caused death. Novy made plate cultures from the cheese and from the spleen and liver of the dead animals, and succeeded in identifying one germ as common to both. Sterilized milk inoculated with a pure culture of this germ, and kept in the incubator, proved fatal to cats. But with the advent of cold weather the germ lost its toxicogenic properties, which were not restored by subsequent cultivation in the incubator.

“In a second class of samples, the poisonous character of the cheese was not confirmed by direct feeding. Cats, rats, and dogs were fed with the same quantities as above, without any appreciable effect. The report made upon the samples was as follows:

“‘Animals fed upon the cheese were not affected. Tyrotoxicon could not be found. The sickness in the people was probably due to some other cause.’

“The last sentence of this report was probably wrong, as will be shown from the following experiment. Two kilograms of a cheese of this class was extracted repeatedly with absolute alcohol. The part insoluble in alcohol was then extracted with water. The aqueous extract, after filtration, was allowed to fall slowly into three times its volume

of absolute alcohol. A voluminous, flocculent precipitate resulted. After twenty-four hours the supernatant fluid was decanted, and the precipitate was dissolved in water and reprecipitated with absolute alcohol; then it was collected and speedily dried on porous plates. A small bit of this precipitate was dissolved in water; and forty drops of this solution, injected under the skin on the back of cats, produced invariably within one hour vomiting and purging. After the partial collapse which followed the vomiting and purging, and which was evidenced by the animal sitting with its chin resting on the floor, recovery gradually followed. The same amount of the solution injected into the abdominal cavity of white rats rendered the animals within ten or fifteen minutes perfectly limp, and the only evidence of life observed was rapid respiratory movements. The rats lay upon their sides, and could be handled without manifesting any attempt at movement. In this condition some died after three or four hours, while others, after lying in this position for from eighteen to twenty-four hours, gradually improved, and after some days seemed to be wholly recovered.

"This substance belongs to the so-called poisonous albumins. From its aqueous solutions it is not precipitated by heat or nitric acid, singly or combined. Its solutions respond to the biuret test. It is not precipitated by saturation with sodium sulphate, nor by a current of carbonic acid gas; therefore, it is not a globulin. It is precipitated by saturation with ammonium sulphate; and this fact removes it from the peptons.

"That animals were not affected when fed with the whole cheese may be explained by the supposition that they did not in this manner get enough of the poison to affect them. It cannot be said positively that the samples of cheese of the first class mentioned above owe their poisonous properties to this substance. We have not had the opportunity of testing samples of this class since the recognition of the poisonous proteid in those of the second class. Four samples of the latter have been tested for the poisonous albumin with positive results.

"It may be found that traces of this poison exist in all samples of green cheese. This point will be investigated.

"It is highly probable that the poisonous effects of some samples of sausage and meat are due to similar products of bacterial activity."

A base isolated from poisonous cheese by LÉPIERRE in 1894 will be mentioned in the chapter on the chemistry of ptomaines.

In reference to the poisonous proteids in cheese and other articles of food the following interesting questions arise: How is the poisoning explained? Is it not generally supposed that poisonous proteids are not absorbable from mucous membranes? MITCHELL and REICHERT showed that the venom of serpents may be absorbed from mucous membranes; especially did they find this to be true of the poisonous pepton of the cobra. ENRIEUX and later RÉPIN have shown that abrin, the poisonous albumose of jequirity, may in part be absorbed by the mucous membrane of the alimentary canal. It may be, however, that the bacteria, which are in the cheese and to which the formation of the poisonous proteids is due, find their way through the intestinal walls and form their poisonous products within the spleen and other organs. The fact that NOVY found the bacteria in the spleen and liver of the animals experimented upon confirms this view.

At noon, February 28th, 1895, a man sixty-seven years old, who had served during the civil war, and since suffered from chronic diarrhoea, took a lunch consisting of cheese, crackers, milk and dried beef. Within an hour he complained of severe pains in the stomach. This increased rapidly in intensity, and a physician was summoned. Ineffectual attempts were made to induce vomiting and move the bowels. At 10 A. M. the next day, death resulted, apparently from exhaustion. The cheese had been in the house for three months, during which time none of it had been used. No postmortem examination was made. The cheese was examined by VAGHAN and PERKINS. The small piece sent for examination was hard and dry. From the interior portions were taken and cultures made under anaërobic conditions. Two bacilli, one

a short rod with rounded ends, length not more than two and one-half times the breadth, the other from three to four times as long as broad, developed. These organisms were isolated by means of Novy's anaerobic plate apparatus. The short bacillus proved fatal to white rats, guinea-pigs and rabbits. The long bacillus was inert. Mixed cultures were poisonous, but not more so than the pure cultures of the short bacillus. One-fourth c.c. of beef-tea and milk cultures from four to seven days old killed all the above mentioned animals within from twenty-four to forty-eight hours. Cultures of the short bacillus had no disagreeable odor, while those of the long one did. Even when the germ was removed by filtration through porcelain, cultures of the short bacillus in quantities of from one to two c.c. killed, thus showing that the organism elaborates a powerful chemie poison, the nature of which has not been determined, but enough is known to say that it is not tyrotoxicon.

The bacillus is more motile and takes the ordinary basic anilin stains readily. Its optimum temperature is that of the body, but at ordinary room-temperature it multiplies and elaborates its poison. Cultures kept in an ice-chest were without effect upon animals. It is a facultative anaerobe. It coagulates milk and decolorizes litmus gelatin. It produces gas, does not liquefy gelatin, and forms a thin, scarcely visible growth on potato.

POISONOUS MILK.—In 1885 VAUGHAN found tyrotoxicon in milk which had stood in a well-stoppered bottle for about six months. It was presumed that this milk was, when first obtained, normal in composition, but, since this was not known with certainty, the following experiments were made: Several gallon bottles were filled with normal milk, tightly closed with glass stoppers, and allowed to stand at the ordinary temperature of the room. From time to time a bottle was opened and the test for tyrotoxicon was made. These tests were followed by negative results until about three months after the experiment was begun. Then the poison was obtained from

one of the bottles. The coagulated milk was filtered through paper. The filtrate, which was colorless and decidedly acid in reaction, was rendered feebly alkaline by the addition of potassium hydrate and agitated with ether. After separation, the ethereal layer was removed with a pipette, passed through a dry filter-paper in order to remove a flocculent white substance which floated in it, and then allowed to evaporate spontaneously. If necessary, this residue was dissolved in water and again extracted with ether. As the ether takes up some water, there is usually enough of the latter left after the spontaneous evaporation of the ether to hold the poison in solution, and in order to obtain the crystals this aqueous solution must be allowed to stand for some hours in vacuo over sulphuric acid.

From one-half gallon of the milk there was obtained quite a concentrated aqueous solution of the poison after the spontaneous evaporation of the ether. Ten drops of this solution placed in the mouth of a small dog, three weeks old, caused within a few minutes frothing at the month, retching, the vomiting of frothy fluid, muscular spasms over the abdomen, and after some hours watery stools. The next day the dog seemed to have partially recovered, but was unable to retain any food. This condition continuing for two or three days the animal was killed with chloroform. No examination of the stomach was made.

In 1886 NEWTON and WALLACE obtained tyrotoxicon from milk and studied the conditions under which it forms. Their report is of so much value that the greater part of it is herewith inserted.

"On August 7th twenty-four persons, at one of the hotels at Long Branch were taken ill soon after supper. At another hotel, on the same evening, nineteen persons were seized with the same form of sickness. From one to four hours elapsed between the meal and the first symptoms. The symptoms noticed were those of gastro-intestinal irritation, similar to poisoning by any irritating material—that is, nausea, vomiting, cramps, and collapse; a few had diarrhoea.

Dryness of the throat and burning sensation in the œsophagus were prominent symptoms.

"While the cause of the sickness was being sought for, and one week after the first series of cases, thirty persons at another hotel were taken ill with precisely the same symptoms as noticed in the first outbreak.

"When the news of the outbreak was published one of us immediately set to work, under the authority of the State Board of Health, to ascertain the cause of the illness. The course of the investigation was about as follows :

"The character of the illness indicated, of course, that some article of food was the cause, and the first part of our task was to single out the one substance that seemed at fault. The cooking utensils were also suspected, because unclean copper vessels have often caused irritant poisoning. Articles of food, such as lobsters, crabs, blue fish, and Spanish mackerel, all of which at times, and with some persons very susceptible to gastric irritation, have produced toxic symptoms, were looked for, but it was found that none of these had been eaten at the time of the outbreak. The cooking vessels were examined, and all were found clean and bright, and no evidence of corrosion was presented.

"Further inquiry revealed the fact that all who had been taken ill had used milk in greater or less quantities, and that persons who had not partaken of milk escaped entirely; corroborative of this, it was ascertained that those who had used milk to the exclusion of all other food were violently ill. This was prominently noticed in the cases of infants fed from the bottle, when nothing but uncooked milk was used. In one case an adult drank about a quart of the milk, and was almost immediately seized with violent vomiting followed by diarrhœa, and this by collapse. Suffice it to say, that we were able to eliminate all other articles of food and to decide that the milk was the sole cause of the outbreak.

"Having been able to determine this, the next step was to discover why that article should, in these cases, cause so serious a form of sickness.

"The probable causes which we were to investigate were outlined as follows: (1) Some chemical substance, such as borax, boric acid, salicylic acid, sodium bicarbonate, sodium sulphate, added to preserve the milk or to correct acidity. (2) The use of polluted water as an adulterant. (3) Some poisonous material accidentally present in the milk. (4) The use of milk from diseased cattle. (5) Improper feeding of the cattle. (6) The improper care of the milk. (7) The development in the milk of some ferment or ptomain, such as tyrotoxin.

"At the time of the first outbreak we were unable, unfortunately, to obtain any of the noxious milk, as that unconsumed had been destroyed; but at the second outbreak a liberal quantity was procured.

"It was soon ascertained that one dealer had supplied all the milk used at the three hotels where the cases of sickness had occurred. His name and address having been obtained, the next step in the investigation was to inspect all the farms, and the cattle thereon, from which the milk was taken. We also learned that two deliveries at the hotels were made daily, one in the morning and one in the evening; that the milk supplied at night was the sole cause of the sickness, and that the milk from but one of the farms was at fault. The cows on this farm were found to be in good health, and, besides being at pasture, were well fed with bran, middlings, and corn-meal.

"So far we had been able to eliminate as causes diseased cattle and improper feeding, and we were then compelled to consider the other possible sources of the toxic material.

"While the inspection of the farms was being made, the the analysis of the milk was in progress. The results of this showed that no chemical substance had been added to the milk, that it was of average composition, that no polluted water had been used as a diluent, and that no poisonous metals were present. This result left us nothing to consider but two probable causes; improper care of the milk, and the presence of a ferment.

"As to the former, we soon learned much. The cows were milked at the unusual and abnormal hours of midnight and noon, and the noon's milking—that which alone was followed by illness—was placed, while hot, in the cans, and then, without any attempt at cooling, carted eight miles during the warmest part of the day in a very hot month.

"This practice seemed to us sufficient to make the milk unpalatable, if not injurious, for it is well known that when fresh milk is closed up in a tight vessel and then deposited in a warm place, a very disagreeable odor and taste are developed. Old dairymen speak of the animal heat as an entity, the removal of which is necessary in order that the milk shall keep well and have a pleasant taste. While we do not give this thing a name, we are fully convinced that milk should be thoroughly cured by proper chilling and aeration before it is transported any distance or sold for consumption in towns or cities.

"This opinion is based on a study of the methods prevalent among experienced dairymen, who ship large quantities of milk to our great cities. The usual practice is to allow the milk to stand in open vessels, surrounded by ice or cold water, for from eight to twelve hours before transportation, and when placed on the cars it has a temperature of from 50° to 60° F., and is delivered to consumers in a perfectly sweet condition. The city of New York receives about 200,000 gallons each day from the surrounding country, and much of it brought in by the railroads has been on the cars from a time varying from six to twelve hours, yet we seldom hear of any of this milk undergoing the peculiar form of fermentation set up in the Long Branch milk. We may account for this by assuming that the proper care of the milk after it was taken from the cow, and the low temperature at which it was kept, have prevented the formation of any ferment; this opinion seems to be indorsed by all dairymen and managers of large creameries with whom we have consulted. They all agree in stating that the milk maintained at a low

temperature can be kept sweet and in good condition for many days.

"We have dwelt on this branch of our topic somewhat extensively, because we are fully persuaded that the improper care of the milk had much to do with the illness it produced.

"The results of our inquiry having revealed so much, we next attempted to isolate some substance from the poisonous milk, in order that the proof might be more evident. A quantity of the milk that had caused sickness in the second outbreak was allowed to coagulate, was then thrown on a coarse filter, and the filtrate collected. This latter was highly acid, and was made slightly alkaline by the addition of potassium hydrate. This alkaline filtrate was now agitated with an equal volume of pure, dry ether, and allowed to stand for several hours, when the ethereal layer was drawn off by means of a pipette. Fresh ether was added to the residuum, then agitated, and, when separated, was drawn off and added to the first ethereal extract. This was now allowed to evaporate spontaneously, and the residue, which seemed to contain a small amount of fat, was treated with distilled water and filtered, the filtrate treated with ether, the ethereal solution drawn off and allowed to evaporate, when we obtained a mass of needle-shaped crystals. This crystalline substance gave a blue color with potassium ferrieyanid and ferric chlorid, and reduced iodic acid. The crystals, when placed on the tongue, gave a burning sensation. A portion of the crystals was mixed with milk and fed to a cat, when, in the course of half an hour, the animal was seized with retching and vomiting, and was soon in a condition of collapse, from which it recovered in a few hours.

"We are justified in assuming, after weighing well all the facts ascertained in the investigation, that the sickness at Long Branch was caused by poisonous milk, and that the toxic material was tyrotoxicon.

"The production of this substance was no doubt due to the improper management of the milk—that is, too long a time was allowed to elapse between the milking and the cooling of

the milk, the latter not being attended to until the milk was delivered to the hotel; whereas, if the milk had been cooled immediately after it was drawn from the cows, fermentation would not have ensued, and the resulting material, tyrotoxin, would not have been produced."

In the same year, SCHEARER found the same poison in the milk used by, and the vomited matter of, persons made sick at a hotel at Corning, Iowa.

In 1887, FIRTH, an English army surgeon stationed in India, reported an outbreak of milk poisoning among the soldiers of his garrison. From the milk he separated, by Vaughan's method, tyrotoxin. He also obtained tyrotoxin from milk which had been kept for some months in stoppered bottles, as had been previously done by Vaughan. (See page 98.)

In 1887, MESIC and VAUGHAN observed four cases of milk poisoning, three of which terminated fatally, and NOVY and VAUGHAN obtained tyrotoxin from the milk and from the contents of the intestine in one of the fatal cases. VAUGHAN reports these cases as follows:

"September 23, 1887, I was visited by Dr. A. G. Mesic, of Milan, Michigan, who informed me that he had four members of a family under his charge, all of whom were seriously ill with peculiar symptoms which he believed to be caused by tyrotoxin. Since Dr. Mesic has written out for me the history of these cases, I will insert his report in full, as follows:

"Saturday, September 17, while passing the residence of S. H. Evans, a respectable farmer, I was called in to see him. I found him—a man about fifty years, spare and muscular—vomiting severely; with flushed face, but with a temperature of 96° F. There was marked throbbing of the abdominal aorta; the tongue had a white, heavy coating, and the breathing was very labored. I set to work with the ordinary remedies to allay the vomiting, which had already continued for some hours. The vomited matters were colored with bile. Pupils were dilated, and a rash resembling that of scarlatina, but coarser, covered the chest, forearms, and legs below the

knees, while the abdomen and thighs remained unaffected. As the bowels had not been moved since the beginning of the attack, I administered a purgative dose of calomel with a little podophyllin and rhubarb. On Sunday a small stool resulted. During that day and night and the following day the retching and vomiting continued. Small doses of carbolic acid seemed to give the most relief. After the movement of the bowels the symptoms were somewhat more promising; but a heavy and unfavorable stupor was observable and persistent.

“On Sunday the coating of the tongue remained very thick, and had changed to a dark brown color. At first I thought that his symptoms indicated a depressed condition, which I had known in one instance to precede typhoid fever. However, after a few days, I concluded that I must look for the cause of the condition among the poisons; but I could think of no one poison which would be likely to produce all the symptoms observed. During Monday, Tuesday, and Wednesday, there was but little change, and the treatment was continued.

“On Thursday morning I found the son Arthur, a lad of eighteen years, strong and vigorous, suffering with the same symptoms, only in a more violent form. After supper on Wednesday evening he was taken with nausea and vomiting. He had no rash, but the symptoms were otherwise identical with those of the father, except in being more severe. I gave a cathartic, which acted only slightly.

“At my evening visit I found Mrs. Evans, a lady of about forty-five, previously in good health, with the same symptoms. In this case the stupor was more marked from the first. I was unable at any time to obtain any cathartic action in this case. Copious enemata of warm water were used, but succeeded only in washing some hardened lumps from the rectum. By this time I had concluded that the poison was most likely tyrotoxinon.

“On Friday morning the only remaining member of the family at home, Miss Alma, sixteen years of age, was affected

in the same way as the others. On that day I went to Ann Arbor, and gave a history of the cases so far to Dr. Vaughan, who, from the symptoms, thought that my diagnosis was most probably correct, and he advised with me as to treatment, which I carried out. I gave two grains of sodium salicylate every four hours, and used small doses of the tonics and stimulants, quinine, *nox vomica*, *digitalis*, whiskey, and the aromatic spirits of ammonia. On Saturday the symptoms in all remained unimproved, and in the mother and son the stupor and labored breathing grew more marked.

“On Sunday I again went to Ann Arbor, and brought Dr. Vaughan with me to see the patients. The temperature of the mother on Sunday was as low as 94° F., and that of the son 95° F. Dr. Vaughan agreed with me as to diagnosis and treatment. Sunday evening the patients were all removed to the house of a neighbor, about forty rods distant (the reasons for this will be given later). Dr. Vaughan and I both expressed the fear that the mother, and possibly the son, would not live through the night. Both of these rapidly grew worse, and the son died at 7:45 A.M. and the mother at 4 P.M., Monday.

“During Monday the daughter rapidly grew worse, and at the time of her mother's death could not be aroused, and practically she remained unconscious from that time on. The father was very weak, but retained his consciousness all the time. Convulsive movements of the limbs had been noticed in the son, but not in the mother. These now became more marked in the daughter, who remained in the heavy stupor, with labored breathing, until 5 P.M. Thursday, when she died.

“Mr. Evans has slowly improved, and now, October 18th, is able to walk about the room. The sodium salicylate, even in the small doses used, seemed to cause severe headache; so apparent was this that the drug was discontinued, and drop doses of amyl nitrite, given every hour, seemed to relieve the pain in the head. His temperature remained below the normal until Thursday, October 14th, when it

reached the normal. After this it was found once as high as 99.°5 F., then 99° F., then again normal, where it remains.

“All complained of a burning constriction in the throat, and difficulty in swallowing, and all, as long as they were conscious, frequently called for ice. In all the pulse was rapid and feeble, and death seemed to result from failure of the heart. Those who died voided urine involuntarily, while Mr. Evans passed small quantities frequently, and for this buchu and uva ursae were given. During his convalescence small doses of morphine were give, as he was unable to sleep, and became very restless. He is now taking teaspoonful doses of the elixir of calisaya and iron every four hours.’

“As stated above by Dr. Mesic, I first saw these patients Sunday, September 25th. On a sofa in the room we found the daughter, Alma. She had been vomiting during the day, and seemed much exhausted. She was not inclined to talk, and seemed to be in a stupor, though when spoken to she responded rationally. Her pupils were slightly dilated, her tongue coated, her pulse 120 and weak, her face flushed, and a violent throbbing could be felt over the abdomen, which was retracted. Her temperature was 96° F.

“In another room were the father, mother, and son, two of them dying. The father was rational and talked with some freedom when I asked as to the kind of food they had been eating, etc. His pupils were normal. His face could not be said to present any peculiar feature. His pulse was rapid, breathing somewhat labored, and the throbbing of the abdominal aorta was plainly felt. The abdomen was retracted, and there was no pain on pressure. He complained of a burning constriction of the throat, swallowed with difficulty, and said that his throat and stomach felt as though they were on fire.

“The mother lay perfectly still with eyelids closed, as if in a deep sleep. Her pulse was rapid, her face had a livid flush, her breathing was about 35 per minute, and labored. The skin was cool, but neither abnormally moist nor specially dry and harsh. She could not be aroused. In fact, she was comatose.

"The son rolled uneasily from one side of the bed to the other. His breathing, also, was very labored. His eyelids were closed, and the pupils were markedly dilated—did not respond to light. He could not be aroused. In mother and son, as well as in father and daughter, the abdomen was retracted, and the throbbing of the abdominal aorta was easily felt.

"Now, to what were these symptoms due? They were certainly those of some poison. Dr. Mesic had brought me some of the vomited matter, which I tested thoroughly for mineral poisons, with negative results. The symptoms certainly were not those of morphin, strychnin, digitalis, or aconite. They did have some resemblance to those of belladonna, but yet they were not the symptoms of belladonna. The pupils were not as widely dilated as they would be in belladonna poisoning. There was in none of these persons the active delirium of belladonna poisoning. There was no picking at the clothing, no grasping of imaginary objects in the air, no hallucinations of vision. Surely it could not be any vegetable alkaloid with which I was familiar.

"On the other hand, we know that nausea, vomiting, headache, dilatation of the pupil, rapid pulse, heavy breathing, constipation, and great prostration, with stupor, do occur in cases of poisoning with certain ptomaines. Therefore we began to look for conditions which would be favorable for the production of putrefactive alkaloids. These conditions we were not long in finding.

"The family, which consisted of the four persons sick, and of a daughter about twenty years of age, who was away from home at the time when the others were taken ill, and for some months before that time, was evidently a tidy one. This was shown by their personal appearance and by the clothing and bedding. But the house in which they lived was very old, and very much decayed. Mr. Evans had purchased the farm six years ago; and for some three years past, at least, they had been troubled every now and then, one or more of the family, with nausea and vomiting, followed by more or less

prostration. But in no instance, up to the present illness, had the symptoms been sufficient to cause them to summon a physician. The family had worked hard in order to pay for the farm, and had determined to make the old house do until they were out of debt. Even before this family had moved to the farm, the house had been known among the neighbors as an unhealthy one, and there had been much sickness and a number of deaths among its former tenants.

“The house is a frame one, and one of the neighbors said to me that it was an old house when he came to the neighborhood thirty-seven years ago. It consists of two rooms on the ground-floor, with attic rooms above. The frame rests upon four large logs or sills, which lie directly upon the ground, and are thoroughly rotten. There is no cellar under any part of the house. From the front, at least, the surface slopes toward the house, and the rain-water runs under it. In the floor of one room a trap-door had been placed, and directly under this a small excavation had been made for the purpose of collecting the rain-water when it accumulated under the house. Although this pit was dry at the time of our examination, its sides and bottom were marked with cray-fish holes, showing that water had stood in it. The floor was laid of unjointed boards, and every time that it was swept much of the filth fell through the cracks, and every time that the tidy housewife scoured and mopped the floor, the water, carrying with it the filth, ran through the crevices, and thus the conditions most favorable for putrefactive changes were brought into existence and maintained.

“One corner of one of the rooms had been transformed into a small room, or buttery, as it was called, and in this, on shelves, the food was kept. On account of the more frequent scouring demanded by that part of the floor enclosed in this buttery, the boards had rotted away, and a second layer of boards had been placed over the original floor. Between these two floors we found a great mass of moist, decomposing matter, the accumulation of years, which the broom could not reach. When this floor was taken up, a peculiar, nauseating

odor was observable, and was sufficient to produce nausea and vomiting in one of the persons engaged in the examination. Some of the dirt from beneath the floor, and some of that which had accumulated beneath the boards in the buttery, were taken for further study.

"The condition of the house was supposed to be unfavorable to the patients, and for this reason they were moved, as Dr. Mesic has stated, to the house of a neighbor. Of course, thorough examination of the house was not made until the patients had been removed.

"Special inquiry was now made concerning the food used by this family. They had been living very simply. They lived upon bread, butter, milk, and potatoes, with coffee and ripe fruit. They had eaten no canned foods for months. They ate but little meat. Occasionally a chicken was killed and served, and rarely, some fresh meat was obtained from the village. During the week in which they were taken ill, all the meat used consisted of slices from a piece of bacon, the only meat which was kept in the house, and a chicken. None of the latter remained, but the bacon was examined. It seemed in perfect condition, and contained no trichinae. Moreover, as has been seen from the history of the cases, all the members of the family were not made sick by any one meal, but the opportunity of obtaining the poison must have been present for some time. Moreover, the fact that previous similar, but less severe, attacks had occurred at intervals for the past three years, convinced us that the poison must owe its origin to some long-existing condition.

"The drinking-water supply was also investigated. The water was obtained from a shallow well, and some of it was taken for analysis. But several families had for years used water from this well, and had remained healthy.

"The milk used by the family was studied. Of course, we could get none of that which had been used before the members of the family were stricken down. As soon as he made the diagnosis of tyrotoxicon poisoning, Dr. Mesic ordered the discontinuance of the use of milk, not only with

the sick, but he forbade the daughter, who had returned, and any of the visitors using it. Mr. Evans owned four milch cows, and they were supplied with fair pasturage and abundant water. The greater part of the milk was placed in tin cans which were set in a wooden trough in the yard, and surrounded by cold water. The covers to the cans were arranged so that the air could have free access to the milk, and were left in this position until the milk was thoroughly cooled. Indeed, the cans, were furnished by a creamery company, which followed the directions I had previously given for the care of milk. On his first visit to me Dr. Mesic brought some of the milk from one of these cans. This I examined, but failed to find tyrotoxicon in it.

"However, the family did not drink any of the milk from the cans. That which they did use was kept in the buttery which I have described. Here it stood upon a shelf, and some members of the family, at least, were in the habit of drinking from it between meals. This was especially true, it is said, of the son. He would frequently come from his work in the fields, go into the buttery and drink a glass or more of the milk. Mr. Evans states that he frequently observed that the taste of the milk was not pleasant. On my first visit to the premises I advised that some of the milk should be taken from the cans, allowed to stand in the buttery over night, and be sent to me the next day. This was done, and in this milk we found tyrotoxicon, not only by the employment of chemical tests, but by poisoning a kitten with it.

"On the death of the mother and son, Dr. Mesic asked for a postmortem, but the friends objected, and the undertaker used an arsenical embalming fluid, so that, although consent was subsequently obtained, it was decided that the examination would be so vitiated as to be worthless. On the death of the daughter, the coroner summoned a jury and held an inquest. The postmortem was conducted by Dr. George A. Hendricks, in the presence of the jury and several physicians who had been invited. Dr. Hendricks has kindly

furnished me with his report, which I present herewith in full :

“The autopsy was held fifteen hours after death. The abdominal viscera were first examined. The great omentum was small, in normal position, covering the small intestine. The small intestine was moderately distended with flatus. The jejunum was ashy-green in color; the ileum purplish-green. About eighteen inches from the termination of the ileum was found a diverticulum two inches in length. The small intestine contained very little alimentary matter. The vermiform appendix was free, contained some small fecal lumps, and showed no evidence of inflammation. The cæcum, ascending, transverse, and descending colon were empty and their circular fibres were tightly constricted, except at intervals where the intestine was distended with gas. The sigmoid flexure was moderately distended with gas, and the rectum contained small bits of fecal matter. The stomach was somewhat contracted and lay wholly upon the left side of the median line. It contained a few ounces of fluid. Its extremities were ligated and the organ removed. The mucous membrane of the stomach and intestine were not examined until they reached the chemist. The duodenum was distended with flatus. The liver was normal in size and appearance. The gall-bladder contained about one ounce of bile. The spleen was normal. One-half ounce of fluid deeply stained with blood was found in Douglas's cul-de-sac. The uterus, Fallopian tubes, and ovaries were deeply congested. The left ovary was enlarged and presented on its posterior surface a hemorrhagic spot, oval, about one-half line in length, and several other less distinct ones. The right ovary was normal in size and showed numerous Graafian scars. The ureters and bladder were normal; the latter contained a small amount of urine. The peritoneum, pancreas, and kidneys were perfectly normal.

“The thoracic cavity was next opened. The lungs were normal; there was about one-half ounce of free serum in the left pleural cavity; none in the right. Pericardium normal;

right auricle in diastole; left auricle and both ventricles in systole.

“The dura mater showed venous congestion; the arachnoid normal; the pia mater congested. On the surface of the centrum ovale, small drops of blood oozed from the divided vessels. The large veins of the velum interpositum were distended. Third and fourth ventricles were slightly distended with serous fluid, but the walls were normal. There seemed to be slight softening of the optic thalami. The sub-arachnoid fluid was about twice the normal quantity.

“On examination of the mucous membrane of the stomach and intestine in the presence of the chemist, Prof. A. B. Prescott, nothing abnormal could be found. The membrane was stained with bile, but there was not the slightest redness. The solitary glands were distinct, but not at all inflamed. Peyer's patches were normal.’

“It will be seen that there existed no lesion which would account for the death. The venous congestion observed in the brain would follow from failure of the heart.

“Some of the postmortem appearances bore a striking resemblance to those which I had observed in cats poisoned with tyrotoxin. This was especially noticeable in the condition of the mucous membrane of the stomach and intestine. Tyrotoxin produces the symptoms of a gastro-intestinal irritant, but not the lesions. The contraction of the circular fibres of the intestine, which undoubtedly caused the constipation, I had also observed in cats that died from tyrotoxin poisoning without either vomiting or stool.¹ The action of this poison upon the stomach and intestine must be through the nervous system. Small doses cause both vomiting and purging, while after large doses vomiting may be impossible, and obstinate constipation may exist. Both the vomiting

¹ MARSH reports a case in which the symptoms resembled very closely those of rapidly perforating appendicitis, but the postmortem examination showed absolutely no evidence of this disease or of peritonitis. In fact the only abnormality found in the intestines consisted of the contraction of the circular fibres of the transverse and descending colon. MARSH believes that this was a case of ptomain poisoning.

and purging after small doses are undoubtedly due in part to increased activity of the circular fibres of the muscular coats, induced through the nerves; and the inability to vomit, and the constipation, one or both of which may be observed after large doses of the poison, are due to spasm of the same muscles, induced in the same manner.

"Prof. A. B. Prescott was requested by the coroner to analyze the material for mineral and vegetable poisons. He made analyses of the stomach and part of its contents, and a portion of the liver. His results were wholly negative.

"Novy tested a cold-water-extract of the finely divided intestine for ptomaines. The fluid, which was acid in reaction, was filtered, then neutralized with sodium bicarbonate, and shaken with ether. The ether, after separation, was removed, and allowed to evaporate spontaneously. The residue was dissolved in water, and extracted again with ether. This ether residue gave the chemical reactions for tyrotoxin, and a portion of it was administered to a kitten about two months old. Within half an hour after the administration the kitten began to retch, and soon it vomited. Within the next three hours it was noticed to vomit as many as five times. The animal sat with its head down, and seemed greatly prostrated. The pupils were examined, but could not be said to be dilated. There was no purging. The retching and heavy breathing, with evidences of prostration, continued more or less marked for two days, after which the animal slowly improved.

"A quantity of fresh milk was divided into five portions of one quart each, placed in quart bottles which had been thoroughly cleansed, and treated in the following manner:

"No. 1 consisted of the milk only, and was employed as a control test.

"No. 2 was mixed with a drachm of vomited matter.

"No. 3 was treated with a portion of the contents of the stomach.

"No. 4 was treated with an aqueous extract of the intestine.

"No. 5 was treated with a small portion of the soil which

had been taken from the floor of the buttery, stirred up with water.

"These bottles were placed in an air-bath, and kept at a temperature of from 25° to 30° C. for twenty-four hours. Then each was tested for ptomaines. No. 1 yielded no tyrotoxicon, while all of the others contained this poison. The tests were both chemical and physiological. All of the samples yielded a non-poisonous base when treated according to Brieger's method, and the same substance was obtained from perfectly fresh milk. It was most probably formed by the action of the heat and reagents employed in this method. This base was obtained in crystalline form, and several portions of it were administered to kittens without any effect. The further study of this body will be of interest to toxicologists, because it gives many of the general alkaloidal reactions. At first we supposed it to be Brieger's neuridin, and this supposition may still be correct, but, as we obtained it, it gave some reactions which are not given by neuridin. Further investigations will be made on this point.

"Tyrotoxicon was obtained from the filtered milk by two methods: (1) The one which we have previously used, and which consists in neutralizing the filtered milk with sodium bicarbonate, and extracting with ether. That portion of the poison employed in the physiological tests was obtained in this way, and in order to be sure that no poison came from the ether, the extract from the milk to which nothing had been added was given to a kitten, and was found to produce no effect. (2) The filtrate from the milk was heated to 70° C. (158° F.)—tyrotoxicon decomposes at 91° C. (195.8° F.)—for some minutes, and filtered. This filtrate, which was perfectly clear, was treated with a small quantity of nitric acid in order to convert the tyrotoxicon into a nitrate, then pure potassium hydrate in the solid form was added until the solution was strongly alkaline. This solution was concentrated so far as it could be on the water-bath. (The potassium compound of tyrotoxicon is not decomposed below 130° C. (234° F.). The dark-brown residue, after cooling, was examined

with the microscope and found to contain the crystalline plates of tyrotoxicon-potassium hydrate, along with the prisms of potassium nitrate. The former was separated from the latter by extraction with absolute alcohol and filtration. The alcohol was evaporated to dryness on the water-bath, and the residue again extracted with absolute alcohol. From this alcoholic solution tyrotoxicon was precipitated with ether. The precipitate was decomposed by adding acetic acid and heating, the tyrotoxicon being broken up into nitrogen and phenol. The phenol was recognized by precipitation with bromine water, and by other well-known tests.

"On October 8th, the coroner's inquest, which had been adjourned after the postmortem in order to await the results of the analysis, was resumed, and after hearing the testimony in accordance with the above-stated facts, the jury returned a verdict of death from poisoning with tyrotoxicon."

CAMMAN reports twenty-three cases of milk poisoning which he attributes to tyrotoxicon, although this poison could not be found in the milk. It may be that the active agent present belongs to the bacterial proteids.

KINNICUTT has isolated tyrotoxicon from milk which had been kept for some hours in an unclean vessel.

GAFFKY reports cases of enteritis due to the use of uncooked milk contaminated with the bacillus coli communis. Two assistants and a servant in Gaffky's laboratory were the sufferers. It is probably of sufficient interest to give some of the details of these cases, because at least one of them might have been mistaken for typhoid fever, and the probabilities are that many cases diagnosed as typhoid fever are actually cases of enteritis, due to food infection. D., the chemist of the Hygienic Institute at Giessen, did not feel well on the morning of October 10th, but in company with assistant B. he visited Frankfort. During the day he had a severe headache, no appetite, and frequent chilly sensations. On returning to Giessen at night he was scarcely able to walk. On the next day he refused all food, was slightly delirious, and had one watery stool. On the 13th his condition

showed serious infection. His face was red, eyes sunken, temperature 41° , and he lay in a half-unconscious state. The tongue was heavily coated; the abdomen distended and painful on pressure. He had five dark-brown, later greenish, stools. The urine was concentrated, and contained two per cent. of albumin, as determined by an Esbach tube. It gave the diazo and indican reactions, and contained white blood-corpuscles and numerous granular casts.

From the 13th to the 17th the patient was stupid, but not delirious. The abdomen was greatly distended, and from twenty to twenty-four stools were passed each day with great tenesmus. The temperature remained high notwithstanding repeated one gram doses of antipyrin. Sleep was broken, and one gram of sulfonal was given at night. The urine remained as before, and the pulse varied from 92 to 100.

From the 18th to the 20th the mental dulness was less marked. Appetite was somewhat improved, and the number of stools decreased to from eight to ten in twenty-four hours. The amount of albumin in the urine was somewhat decreased and microscopic examination showed fatty casts and white blood corpuscles. Small doses of opium were given by mouth and in suppositories.

On the morning of the 21st hemorrhage from the bowels, about 300 c.c. in amount, occurred. Several doses of opium were given, and ice-bags were kept on the abdomen. After the hemorrhage there were three slightly bloody stools. From this time, the improvement was slow but fairly constant. The fever disappeared October 29th.

After recovery, marked anæsthesia of the anterior surface of the thigh developed and remained for some weeks, the anæsthetic area gradually becoming smaller. The hair fell out, mental activity tired, and the eyes were easily fatigued for some months.

The other cases were less severe and less prolonged. The only food or drink which these three men had in common was some uncooked milk taken on the morning of October 9th. D. ordered the milk sent to the laboratory, drank the

greater part of it himself, giving B. a small cupful, and the servant drank a little left by the others. The cow that gave this milk was suffering from a bloody diarrhœa. GAFFKY found in the stools of the cow and in those of the patients a small highly virulent form of the bacillus coli, and to this he ascribed the ill effects. He supposes that some of the liquid discharges from the cow fell upon the udder, and thus found its way into the milk.

GAFFKY suggests that the epidemic in Christiana in 1888, in which 6,000 persons sickened within three weeks, was probably due to milk infection. HUSEMANN states that this epidemic was regarded as a morbus sui generis. It was evidently neither cholera nor typhoid fever. Half of those affected were children, and yet nurslings escaped altogether. However, milk, as an etiological factor, seems not to have been considered.

REHN also reports a case of continued fever resembling typhoid due to infection of milk with the bacillus coli communis. The patient was a child two and one-half years old. The first noticeable symptom to appear was a mucous diarrhœa. The milk was immediately discontinued, but the child improved so rapidly that the trouble was supposed to be due to some trivial cause and the use of the milk was resumed. The diarrhœa promptly returned and was accompanied by a fever lasting three weeks. After the subsidence of the fever, boils appeared on different parts of the child's body, and some months elapsed before the usual health was restored. The bacillus was found in the milk and the stools. It is worthy of observation that the diazo reaction was given by the urine in both GAFFKY's and REHN's case.

Further information on poisonous milk will be found in the sections devoted to the summer diarrhœas of infancy.

POISONOUS ICE-CREAM.—In 1886, VAUGHAN and NOVY obtained tyrotoxicon from a cream which had seriously affected many persons at Lawton, Michigan. Vanilla had been used for flavoring, and it was supposed that the ill-effects

were due to the flavoring. This belief was strengthened by the fact that a portion of the custard was flavored with lemon, and the lemon cream did not affect any one unpleasantly. Fortunately some of the vanilla extract remained in the bottle from which the flavoring for the ice-cream had been taken, and this was forwarded to the chemists. Each of the experimenters took at first thirty drops of the vanilla extract, and, no ill-effects following this, one of them took two teaspoonfuls more with no result. This proved the non-poisonous nature of the vanilla more satisfactorily than could have been done by a chemie analysis.

Later it was found that that portion of the custard which had been flavored with lemon was frozen immediately; while that portion which was flavored with vanilla, and which proved to be poisonous, was allowed to stand for some hours in a building, which is described as follows by a resident of the village:

"The cream was frozen in the back end of an old wooden building on Main Street. It is surrounded by shade, has no underpinning, and the sills have settled into the ground. There are no eave-troughs, and all the water falling from the roof runs under the building, the streets on two sides having been raised since the construction of the house. The building had been unoccupied for a number of months, consequently had had no ventilation, and what is worse, the back end (where the cream was frozen) was last used as a meat market. The cream which was affected was that portion last frozen; consequently it stood in an atmosphere like that of a privy vault for upward of an hour and a half or two hours before being frozen."

The symptoms observed in these cases are given by Dr. MOFITT as follows:

"About two hours after eating the cream everyone was taken with severe vomiting, and after from one to six hours later with purging. The vomit was of a soapy character, and the stools watery and frothy. There was some griping of the stomach and abdomen, with severe occipital headache,

excruciating backache, and bone pains all over, especially marked in the extremities. The vomiting lasted from two to three hours, then gradually subsided, and everybody felt stretchy, and yawned in spite of all resistance. The throats of all were œdematous. One or two were stupefied; others were cold and experienced some muscular spasms. A numb feeling, with dizziness and momentary loss of consciousness, was complained of by some. Temperature was normal, and pulse from 90 to 120. Tongue dry and chapped. All were thirsty after the vomiting subsided, and called for cold water, which was allowed in small quantities, with no bad results. After getting out no one of the victims was able to be in the hot sun for several days, and even yet (about ten days after the poisoning) the heat affects myself. I attended twelve persons, besides being sick myself, and all were affected in pretty much the same way. Several complain yet of inability to retain food on the stomach without distressing them. The man who made the cream took a teaspoonful of it, and he vomited the same as those who took a whole dish, but not so often or for so long a time. All are affected with an irresistible desire to sleep, which can scarcely be overcome. Even yet, some of us feel that drowsy condition, with occasional occipital headache."

The tyrotoxicon obtained from this cream was administered to a kitten about two months old. Within ten minutes the cat began to retch and soon it vomited. This retching and vomiting continued for two hours, during which the animal was under observation, and the next morning it was observed that the animal had passed several watery stools. After this, although the kitten could walk about the room, it was unable to retain any food. Several times it was observed to lap a little milk, but on doing so it would immediately begin to retch and vomit. Even cold water produced this effect. This condition continuing, after three days the animal was placed under ether and its abdominal organs examined. Marked inflammation of the stomach was supposed to be indicated by the symptoms, but the examination

revealed the stomach and small intestine filled with a frothy, serous fluid, such as had formed a portion of the vomited matter, and the mucous membrane very white and soft. There was not the slightest redness anywhere. The liver and other abdominal organs seemed normal.

A bit of the solid portion of this cream was added to some normal milk, which by the addition of eggs and sugar was made into a custard. The custard was allowed to stand for three hours in a warm room, after which it was kept in an ice-box until submitted to chemie analysis. In this tyrotoxon was also found.

Tyrotoxon has since been found in some chocolate cream which poisoned persons at Geneva, N. Y.; and in lemon cream from Amboy, Ohio.

SCHEARER reports the finding of tyrotoxon in both vanilla and lemon ice-cream which made many sick at Nugent, Iowa.

ALLABEN reports poisoning with lemon cream, and makes the following interesting statements concerning it :

"I would first say July 4th, 5th, and 6th were very warm. Monday evening, July 5th, the custards were cooked, made from Monday morning's cream and Monday night's milk, boiled in a tin pan that had the bright tin worn off. It was noticed that one pan of cream was not sweet, but, thinking it would make no difference, it was used; the freezers were thoroughly cleaned and scalded, and the custards put in the same evening while hot; the cream was frozen Tuesday afternoon, having stood in the freezers since the night before, when the weather was very warm."

No analysis of this cream was made, but the symptoms agree with those of tyrotoxon poisoning.

WELFORD observed several cases of poisoning from custard flavored with lemon. These custards were tested for mineral poisons, with negative results.

MORROW has put forth the claim that ice-cream poisoning is solely due to artificially prepared vanillin, which is, according to his statement, used instead of vanilla extract, but the

facts stated above concerning poisoning with creams in which other flavors had been used contradict this claim. Moreover, GISSON has shown the utter absurdity of the claim, inasmuch as he calculates from the amount of flavoring ordinarily used in ice-cream that, in order to produce the toxic symptoms observed, the flavoring must be ten times as poisonous as pure strychnin.

BARTLEY suggests that poisonous cream sometimes results from the use in its manufacture of poor or putrid gelatin. This is highly probable, and with the gelatin the germs of putrefaction may be added to the milk.

PERKINS has recently (August, 1895) isolated an exceedingly virulent germ from some cream that had poisoned a large number of people at Waters, Michigan.

POISONOUS MEAL AND BREAD.—Reference has already been made to the fact that the peasants in certain parts of Italy are frequently poisoned by eating mouldy corn-meal. As has also been stated, LOMBROSO and others have obtained from this meal ptomaïns, some of which gave the same color reaction as strychnin. In 1886, LADD succeeded in isolating from "heated" corn-meal a ptomaïn which forms in urea-like crystals. The quantity was not sufficient for an ultimate analysis, and the physiologic action has not been studied. Poisoning from decomposed and mouldy bread is not unknown.

CHAPTER IV.

THE EXAMINATION OF POISONOUS FOODS.

CHEMISTS and bacteriologists are now frequently asked to examine foods which are suspected of having caused untoward results. Reports of illness due to infected food have become quite frequent in recent years. The increase in the number of cases of this kind is partly real and partly only apparent. One cause of the actual increase is due to the larger consumption of preserved foods. Meats, the appearance and odor of which would render their sale in the piece impossible or at least doubtful, may be chopped, cooked, canned, and sold as first-class articles. We do not intend to state that this fraud is commonly practised, but that it is a possible one cannot be denied, and that it has occasionally been resorted to has been demonstrated both in this country and in Europe. This source of danger to the public health will remain until the necessity of the scientific inspection of foods, especially that of animals before slaughtering, is understood and practised. However, the greater number by far of cases of poisoning by prepared foods arises from imperfections in methods or from want of intelligent and conscientious attention to details.

When we recognize that the successful preparation of every portion of preserved food depends upon the exclusion of micro-organisms, both specific and putrefactive, and when we learn that the processes are carried out for the most part by those who are ignorant of the scientific principles involved, then we can only wonder that the health of the consumer is not more frequently placed in jeopardy.

The apparent increase in the number of instances of food-poisoning is due to the fact that the medical profession has

recently learned to recognize food-infection as a cause of illness, or at least has been in possession of the knowledge necessary to convert suspicion into positive demonstration. Only a few years ago we were seeking for the cause of cholera infantum in mysterious and indefinite telluric or meteorologic conditions, but now we know that this disease is solely due to infected, and, consequently, poisonous food. Formerly, many of these cases were supposed to be due to the accidental or criminal addition of some metallic or vegetable poison to the food, and unjust accusation, possibly in some instances, unjust execution, has resulted. Again, we now know that typhoid and typhus fevers, scarlet fever, and other acute exanthemata, and even pneumonia, may be closely simulated by the symptoms due to infected foods.

Unfortunately the expression "ptomain-poisoning" has come into quite general use to designate cases of illness arising from infected food. While it is true that basic substances of bacterial origin constitute in some instances the actual *materies morbi*, this is not always, or even generally, the truth. Among the poisonous bacterial products there are many which are not basic, and concerning the chemie nature of which we are yet very much in ignorance. In a large proportion of the instances we are ignorant not only of the chemistry of the poisons which induce the untoward effects, but of the bacteria through the activity of which these poisons are generated. Moreover, we cannot in cases of food-poisoning draw a sharp line of distinction between intoxication and infection. Food-poisoning may originate in any one of the following ways: (1) The food is infected and the poison is generated only and wholly before the food is taken. (2) The infecting organism may begin the elaboration of its poisonous products outside of and continue the same process inside the body. (3) The infection may not result in the production of poisons until the food is taken into the body. In all of these forms infection of the food is the essential element. It is this that must be prevented, and to this especial attention must be called.

How shall one proceed in the examination of food suspected of having caused sickness or death?

In the first place, the possibility of the ill-effects having been due to metallic poison should be considered. In cases in which this possibility exists such poisons should be sought by the methods given by the best toxicologists, and which it is not the purpose of this book to repeat. In case the substance to be examined consists of canned food the tests for mineral poisons should always be made. However, when a teaspoonful of ice-cream causes nausea and vomiting, the idea that these effects can be due to sulphate of zinc dissolved in the freezer is too preposterous and absurd to be worthy of serious consideration by any one familiar with the quantity of this salt necessary to produce such effects.

The examination of foods for bacterial poisons cannot be made except in a properly equipped bacteriologic laboratory. It is our purpose to merely point out at this time the methods that may be followed. We take it for granted that the one who undertakes work of this kind is already familiar with the ordinary technique of bacteriological research. The line of procedure will vary somewhat with the kind of food to be examined, the form in which it has been prepared, and the quantity supplied the analyst. All samples should be examined with as little delay as possible after the article has become the object of suspicion. When delay is unavoidable, further bacterial growth should be retarded in the meantime as far as is possible; not by antiseptics, but by low temperature. Germs not present at the time of the supposed poisoning may be accidentally introduced, or non-toxicogenic bacteria may multiply to such an extent that the detection of the harmful organism is rendered impossible.

As a rule, the quantity of the food supplied the analyst is not sufficient to allow of the detection or the isolation of the chemie poison directly. To try to find the poison in a few ounces of cheese or a small bit of meat by direct extraction is a task that would be undertaken only by one quite ignorant of the nature of these poisons. In all but exceptional instances,

where many pounds of the food are supplied, the portion that reaches the laboratory can only be regarded as the bearer of the germ, to the activity of which the poison is due. This germ must be detected, isolated, grown in pure cultures, and its toxicogenic properties demonstrated upon lower animals. It should be clearly understood that the most thorough study of the morphologic characteristics of the germ and of the chemic properties of the poison will not suffice without an accompanying determination of the toxicologic action of the culture. The infectious nature of the bacterium should also be studied.

It should always be borne in mind that the article of food has probably been through several hands, some of which may not have been germ-free. In the examination of pieces of meat and cheese, the surface should be sterilized with a broad heated knife or other piece of iron. It has been shown that bacteria deposited on such surfaces penetrate slowly. Then with other sterilized knives sections should be made, and one or more small bits taken from the interior should be placed in sterilized bouillon. Not less than a dozen tubes should be inoculated in this way. Three of these should be grown aerobically at ordinary temperature; three anaerobically at the same temperature; three aerobically at 37° ; and three anaerobically at 37° . It is quite essential that all these conditions of growth should be tried. Some of the toxicogenic germs grow best at relatively low temperatures (20° to 25°) and fail to develop at 37° . Others have their optimum growth at the last mentioned temperature. Some develop only when the air is excluded, and others only when freely supplied with air.

In the examination of liquid and semi-liquid foods, such as milk, custard, cream, broths, and jellies, small bits or a few drops should be placed in sterilized bouillon and grown under the conditions mentioned above.

A growth having appeared in one or more of these tubes, the bacteria should be examined in hanging drops and in stained mounts. If more than one organism be present, plate

cultures should be made, and each germ should again be grown under the conditions mentioned.

The infectious character of each organism should be tested on the lower animals (1) by feeding, (2) by subcutaneous inoculation, (3) by intraperitoneal inoculation, and (4) by intravenous inoculation. The animals generally employed in these experiments are white mice, white rats, guinea-pigs, kittens, and rabbits. A given germ may be toxicogenic to one of these animals and not to the others. Mice and kittens are specially suitable for feeding experiments. Young kittens are quite susceptible to most of the bacterial poisons found in milk and its products. The quantity of the bouillon-culture—twenty-four hours old or older—first employed should be relatively large—from 1 to 10 c.c., according to the animal and the method of infection. If these amounts prove active, smaller quantities should be tried until the limit is reached.

Next, the action of cultures from which the bacteria have been removed by filtration through porcelain should be tested, and if these prove active the effect of different degrees of heat on the toxicity of the cultures should be determined.

If by the above-mentioned experiments a toxicogenic germ has been discovered, its morphologic, cultural, tinctorial, and pathogenic properties may be studied as thoroughly as the investigator may desire. The study of the bacterial poison may also be carried to the same extent. The examination for ptomaines and toxins can be carried out according to the methods described in subsequent chapters.

CHAPTER V.

GENERAL CONSIDERATIONS OF THE RELATION OF BACTERIAL POISONS TO INFECTIOUS DISEASES.

IN our introduction we have divided diseases etiologically into the following classes: (1) Bacterial; (2) Fungous; (3) Protozoal; (4) Animal parasitic; (5) Intoxications; (6) Traumatic; and (7) Autogenous. The first four of these may be grouped together under the term infectious. It must be understood, however, that in many diseases the cause is not single, but multiple, and for this reason sharp lines of classification cannot be drawn. For instance, the greatest danger in those traumatic affections in which the traumatism itself does not cause death lies in infection. The wound has simply provided a suitable point of entrance for the infecting agent. Indeed, the break in the continuity of tissue may be so slight that it is of import and danger only on account of the coincident infection. This is true in many cases of tetanus. Furthermore, an infectious disease, whether it originates in a traumatism or not, is markedly influenced by what we are pleased to call the idiosyncrasy of the patient, and by which we mean the peculiarities of tissue metabolism taking place in the individual at the time. A dozen men may be exposed alike to the same infection, and the infecting agent may find a suitable soil for its growth and development in two of these, while in the other ten this same agent meets with such adverse influences that it dies without producing any appreciable effects; or all may be infected, but with differences in degree, as is evidenced by variation in symptoms, in the length of time that this infecting agent continues to grow and develop in the body and in the ultimate result. Every physician who has had experience in the treatment of typhoid fever, diphtheria,

scarlet fever, or, in short, of any of the infectious diseases, will appreciate the importance of the personal equation in his patients.

CHARRIN and ROGER have shown that white rats, which are naturally immune to anthrax, become susceptible when fatigued by being kept on a small treadmill. Eleven rats were inoculated with an anthrax culture; five of these which were allowed to rest in the cage manifested no symptoms of the disease, while six which were placed on the tread-mill developed the disease and died within from twenty-four to thirty hours. The bacilli were found in the liver and spleen of those that died; and guinea-pigs inoculated with these germs died. The influence of the condition of health on susceptibility to the infectious diseases has also been shown by LEO, who found that mice, which are naturally insusceptible to glanders, become highly susceptible when they are rendered diabetic by the administration of phloridzin.

That some neurotic affections originate from traumatism we know. That others of this class are largely due to malnutrition accompanied by improper metabolism or insufficient elimination, or, in other words, are to some extent autogenous, all believe. Understanding, then, that the above classification does not attempt a sharp and marked differentiation of the causes of disease, we will now give our attention to a consideration of the chemical factors in the causation of the infectious diseases, and of the traumatic and autogenous, in so far as these are influenced by infection.

Recognizing the fact that germs do bear a causal relation to some diseases, the question arises, How do these organisms produce disease? In what way does the bacillus anthracis, for instance, induce the symptoms of the disease and death? Many answers to this question have been offered. Some of the most important of these are as follows:

1. It was first suggested by BOLLINGER that apoplectiform anthrax is due to deoxidation of the blood by the bacilli. The germs are aerobic, and were supposed to deprive the red blood corpuscles of their oxygen. This theory was suggested

most probably by the resemblance of the symptoms to those of carbonic-acid poisoning. The most prominent of these symptoms are dyspnoea, cyanosis, convulsions, dilated pupils, subnormal temperature, and, in general, the phenomena of asphyxia. Moreover, postmortem examination reveals conditions similar to those observed after death by deprivation of oxygen. The veins are distended, the blood is dark and thick, the parenchymatous organs are cyanotic, and the lungs hyperæmic. BOLLINGER compared this form of anthrax to poisoning with hydrocyanic acid, which was then believed to produce fatal results by robbing the blood of its oxygen.

This theory was supported by the observations of SZPILZMANN, who found that while the putrefactive bacteria are destroyed by ozone, the bacillus anthracis thrives and multiplies in this gas.

This theory presupposed a large number of bacilli in the blood, and this accorded with the estimate of DAVAINE, which placed the number at from eight to ten million in a single drop. But more extended and careful observation showed that the blood of animals dead from anthrax is often very poor in bacilli. VIRCHOW reported cases of this kind. BOLLINGER himself found the bacilli often confined to certain organs and not abundant in the blood. Then SIEDAMGROTZKY counted the organisms in the blood in various cases, and found not only that the estimate made by DAVAINE is too large, but that in many instances the number present in the blood is small. JOFFROY found in some of his inoculation experiments that the animals died before any bacilli appeared in the blood. These and other investigations of similar character began to cause workers in this field of research to doubt the truth of the theory of BOLLINGER, and these doubts were soon converted into positive evidence against it. PASTER, in support of the theory, reported that birds were not susceptible to anthrax, and he accounted for this by supposing that the blood corpuscles in birds do not part with their oxygen readily. However, it was shown by OEMLER and FESER that the learned Frenchman had generalized from limited data, and

that many birds are especially susceptible to the disease. OEMLER found that the blood, even when rich in bacilli, still possesses the bright-red color of oxyhaemoglobin. TOEPPER and ROLOFF reported cases of apoplectiform anthrax in which there was no difficulty in respiration. TOUSSAINT caused animals which had been inoculated with the anthrax bacillus to breathe air containing a large volume of oxygen, and found that this did not modify the symptoms or retard death. Finally, NENCKI determined the amount of physiological oxidation going on in the bodies of animals sick with anthrax by estimating the amount of phenol excreted after the administration of one gram of benzol, and found that the oxidation of the benzol was not diminished by the disease. Thus, the theory that germs destroy life by depriving the blood of its oxygen has been found not to be true for anthrax, and if not true for anthrax, certainly it cannot be for any other known disease. The bacillus anthracis is, as has been stated, aerobic, while many of the pathogenic bacteria are anaerobic—that is, they live in the absence of oxygen. This element is not necessary to their existence, and, indeed, when present in large amount, it is fatal to them. Moreover, in many diseases, the bacteria are not found in the blood at all. Lastly, the symptoms of these diseases are not those of asphyxia. These facts have caused all bacteriologists to acknowledge that this theory is not the right one.

2. If a properly stained section of a kidney taken from a guinea-pig, which has been inoculated with the bacillus anthracis, be examined under a microscope, the bacilli will be found to be present in such large numbers that they form emboli, which not only close, but actually distend the capillaries and larger bloodvessels, and interfere with the normal functions of the organ. A similar condition is sometimes found on microscopical examination of the liver, spleen, and lungs. From these appearances, it was inferred by BOLLINGER that the bacilli produce the diseased condition simply by accumulating in large numbers in these important organs, and

mechanically interrupting their functions. This is known as the mechanical interference theory.

KLEBS and TOUSSAINT were formerly ardent advocates of this theory in its application to anthrax, and the latter thought that the symptoms and death are due to stoppage of the pulmonary circulation by means of emboli. However, HOFFA studied this point by making numerous postmortem examinations, and was unable to confirm it. A like result followed the work of VIRCHOW, COLIN, and SIEDAMGROTZKY, and the mechanical interference theory has been abandoned.

In the majority of germ diseases this theory never had any support. There is not found any great accumulation of bacteria in any organ, and the number and distribution of the germs are such that the theory of mechanical interference cannot be held.

3. Another answer given to the question, How do germs cause disease? is, that they do so by consuming the proteids of the body and thus deprive it of its sustenance. The proteids are known to be necessary for the building up of cells, and it is also known that microorganisms feed upon proteids. But this theory is untenable for several reasons. In the first place, many of the infectious diseases destroy life so quickly that the fatal effects cannot be supposed to be due to the consumption of any very large amount of proteids. In the second place, the distribution of the microorganisms is such in many diseases that they do not come in contact with any large proportion of the proteids of the body. In the third place, the symptoms of the majority of these diseases are not those which would be produced by withdrawing from the various organs their food. The symptoms are not those of general starvation.

4. Still another theory, which has been offered, is that the bacteria destroy the blood corpuscles, or lead to their rapid disintegration. But in many of the infectious diseases, as has been stated, the microorganisms, although very abundant in some organs, are not present in the blood. Moreover, the dis-

integration of the blood corpuscles is not confirmed by microscopical examination.

5. Seeing the vital deficiencies in the above theories, and being impressed by the results obtained by the chemie study of putrefaction, bacteriologists have been led to inquire into the possibility of the symptoms of the infectious diseases being due to chemie poisons. In investigating this theory, three possibilities suggest themselves:

(a) The microorganisms themselves may be poisonous, or the poison may be an integral part of them. NEESEN, at one time an advocate of this theory, thus accounted for the appearance and increase in violence of the symptoms as the germs increase in number. In order for the conditions of this theory to be fulfilled the microorganisms must be present in the blood before any of the symptoms appear. But in anthrax, the most thoroughly studied of all the infectious diseases, and the one to which all these theories have been applied, the bacilli first appear in the blood, as a rule, only a few hours before death, and long after the appearance of the first symptoms; while in many other diseases the germs are never found in the blood. Moreover, as HOFFA has shown, if this theory be true, the injection of a large quantity of anthrax bacilli directly into the blood should be followed immediately by symptoms of the disease, and death should be speedy. But he found, on making experiments of this kind, that the symptoms did not appear until from twenty-four to seventy-two hours. NENCKI found by analysis that the substance of the anthrax bacilli resembles vegetable casein in some respects, and animal mucin in others. This "anthrax protein" is freely soluble in alkalis, is insoluble in water, acetic acid, and the dilute mineral acids. It contains no sulphur and was believed by NENCKI to be inert; but the recent researches of BUCHNER have shown that this belief is not well founded. It has been stated by a number of investigators that suppuration might be induced by the injection of certain sterilized cultures, but the dictum of WEIGERT, "no suppuration without bacteria," has been generally accepted;

and statements to the contrary, although some of them have been made by men of excellent reputation, have until recently received but little credence. However, BUCHNER has shown conclusively that the albuminate of the bacterial cell in as many as seventeen different species possesses well-marked pyrogenetic properties, and that the pus formed is free from germs. BUCHNER separated the microorganisms from the soluble substances accompanying them by sedimentation and decantation, washed the cells, dissolved them in 0.5 per cent. solution of potash by the aid of heat, precipitated the albumin with dilute mineral acid, and, after repeated resolution in alkali and reprecipitation with acid, employed the purified proteid in his experiments. Introduced with antiseptic precautions under the skin, this substance invariably causes supuration. This demonstrates that the substance of the bacterial cell is not altogether inert. It is impossible at present to say to what extent the course of an infectious disease may be influenced by the breaking down of a large number of bacterial cells and the introduction of their substances into the blood; but it can be considered as an established fact that the specific toxins are formed within the bacterial cells. As the cells break down these toxins pass into solution and are carried by the blood and lymph to all parts of the body. If in HOFFA's experiments referred to above, the bacterial cell had been dissolved or broken before being injected, the results would have been different.

(b) The microorganisms may be intimately associated with or may produce a soluble, chemie ferment, which, by its action on the body, produces the symptoms of the disease and death. This theory formerly had a number of ardent supporters, among whom might be mentioned the eminent scientist, DE BARY. But PASTEUR proved the theory false when he filtered anthrax blood through earthen cylinders, inoculated animals with the filtrate, and failed to produce any effect. NENCKI made a similar demonstration when he inoculated a two per cent. gelatin preparation with the anthrax bacillus, which liquefied the preparation, and on stand-

ing the bacilli settled to the bottom. The supernatant fluid, which was clear, alkaline in reaction, and contained dissolved "anthraxprotein," was filtered and injected into animals without producing any effect.¹

It must not be inferred from the above statements that bacteria do not produce any ferments. Many of them do form both diastatic and peptic ferments, which may retain their activity after the bacteria have been destroyed; but there is no proof that in any case these ferments have any causal relation to the disease. After the disease process has been inaugurated some of these ferments probably play an important part in the production of morphologic changes, the nature of which will be indicated when these diseases are discussed.

(c) Poisons may be produced by the cellular activity of the bacteria much in the same way as morphin is formed in the poppy. This theory finds, in the first place, strong support in the well known fact that many of the putrefactive germs produce highly poisonous bodies; and, in the second place, the formation of chemic poisons will account for the appearance of the symptoms of the disease when the living microorganisms never find their way into the blood. The correctness of this theory has been tested by a large number of investigators, and with the result that its truth has been firmly established. It was soon found that pathogenic germs grown in meat broth and other culture media elaborate chemic poisons which, when injected into the lower animals, induce in an acute form one or more of the symptoms characteristic of the disease caused in man by the microorganism. It is true that until quite recently this theory has been opposed by some, and it is altogether possible that at present there may be those who are not altogether convinced of its truth. However, we are not acquainted with any argument against it which remains unanswered. For a while BAUM-

¹ We now know that if the supernatant fluid used in this experiment had been injected in sufficient quantity death would have been produced by the soluble chemic poisons.

GARTEN claimed that the formation of chemie poisons in the dead matter of meat broth and other media by the germ does not prove that the same agent is capable of forming the same or similar products within the living body; but the isolation of tetanin from the amputated arm of a man with tetanus, by BRUEGER, furnished the first positive answer to this criticism, and since that time a number of bacterial poisons have been obtained from the bodies of men and the lower animals. We now expect to find each specific, pathogenic microorganism producing its characteristic poison or poisons. The evidence on this point will be given further on in a brief sketch of the chemie factors in the causation of some of the best known infectious diseases.

Before taking up the individual diseases, we will give what appears to us, in the present state of our knowledge, a correct definition of an infectious disease.

An infectious disease arises when a specific, pathogenic microorganism, having gained admittance to the body, and having found the conditions favorable, grows and multiplies, and in so doing elaborates a chemie poison which induces its characteristic effects.

In the systemic infectious diseases, such as anthrax, typhoid fever, and cholera, this poison is undoubtedly taken into the general circulation, and affects the central nervous system. In the local infectious diseases, such as gonorrhœa, and infectious ophthalmia, the principal action of the poison seems to be confined to the place of its formation. Though even in these, when of a specially virulent type, the effects may extend to the general health. It may be that in some diseases the chemie poison has both a local and a systemic effect. Thus, it is by no means certain that the ulceration of typhoid fever is due directly to the living bacillus. On the other hand, it is altogether probable that the anatomic changes in the intestine result from the irritating effects of the poison at the place of its formation.

With the proof that the deleterious effects wrought by germs are due to chemie poisons elaborated by them during

their growth admitted, let us inquire what properties a micro-organism must possess before it can be said to be the *specific* cause of a disease. The four rules of Koch have been generally conceded to be sufficient to show that a given germ is the *sole* and sufficient cause of the disease with which that germ is associated. Briefly, these rules are as follows:

1. The germ must be present in all cases of that disease.
2. The germ must be isolated from other organisms and from all other matter found with it in the diseased animal.
3. The germ thus freed from all foreign matter must, when properly introduced, produce the disease in healthy animals.
4. The microorganism must be found properly distributed in the animal in which the disease has been induced.

We will briefly discuss the applicability of these rules. When it is stated that the germ must be present in all cases of the disease, it must not be understood that an unlimited number of cases must be examined before the causal relation of a given organism to the disease may be reasonably suspected. This would require more than a lifetime, and would demand facilities for the study of the special disease that do not and cannot exist. The number of cases in which the germ is constantly found should be reasonably large, and the larger this number the greater the probability that the organism is etiologically related to the disease. Moreover, the germ may be present in all cases and yet it may not be found in all. To demand that it be found in all cases would be to presume that the methods of detecting and recognizing a given germ are perfect. There is no ground for this assumption. Then, again, since the results of no one man's work can be accepted in science, until they have been confirmed by others, the personal equation must be considered. What one man finds, another may fail to find. Diligence, skill, and accuracy are not equally developed in all men. The methods employed may differ. To illustrate these points: Koch, after the most painstaking study embracing twenty-nine cases of pulmonary tuberculosis, nineteen of miliary tuberculosis, twenty-one of tuberculous glands, thirteen of tuberculous joints, ten of

tuberculosis of bones, four of lupus, and seventeen of bovine tuberculosis, announced a given bacillus as constantly present in tubercular disease. Since this announcement thousands of physicians and bacteriologists, of different degrees of skill, and often by different methods of staining, with microscopes of all kinds, good and bad, have sought for this bacillus. Is it strange that now and then some man fails to find this bacillus in a genuine case of tuberculosis?

Another most important point in this connection lies in the fact that the clinical and the bacteriological diagnosis do not always agree. The most skilful clinicians may differ concerning a case of membranous sore-throat. One is sure that it is diphtheria; a second is in doubt and reports it as a suspicious or doubtful case; and a third is sure that it is not diphtheria. A bacteriological examination may reveal or fail to reveal the presence of the LOEFFLER bacillus. Again, it may be that any number of the most competent clinicians agree in saying that the case is or is not one of diphtheria, and yet a bacteriological examination may result in a contradictory diagnosis. This is exactly what has happened in the study of diphtheria. From statistics gathered by NOVY it appears that of 8186 cases of clinical diphtheria, diagnosed as such by different men in Europe and America (to May, 1895), the LOEFFLER bacillus was found in 5943, the bacteriological examinations also being made by different men. The clinical diagnosis was confirmed in 72.6 per cent. of these cases. On the other hand, of 333 cases diagnosed as diphtheria by BAGINSKY, 332 furnished the bacillus, and of 117 seen by KOSSEL, all were confirmed by the bacteriological examination. These figures are given to illustrate the factors of variation that may arise in the application of the first of Koch's rules.

Shall we accept the clinical or the bacteriological classification of disease? There can be no doubt that the latter is the more exact, and its adoption will lead to a more accurate and scientific study of disease. An etiological classification of the infectious diseases is one of the great desiderata. Whether it will ultimately be made upon the morphologic characters of

the bacteria or on their poisonous products, cannot yet be determined. There are certain objections to making the first of these the basis that seem at present wellnigh insuperable. Some of these will be discussed later.

The importance of the first of Koch's rules is self-evident. However, the invariable presence of any germ in a certain disease does not prove that the former is the cause of the latter. Indeed, so long as the investigation goes no further than this, we are justified in saying that the microorganism may be an accompaniment or a consequence of the disease; therefore additional evidence is wanting and is furnished by complying with the other rules of Koch.

The second rule is complied with by means of plate and other cultures, a description of which would be out of place here.

The third and fourth rules are difficult of application, because the lower animals are often immune to many of the diseases to which man is susceptible.

In all cases, we insist that the true test of the specific character of a germ is to be made with its chemie products. A given bacterium may not multiply in the circulating blood of a dog, and failure to do so is by no means proof that the same organism might not cause disease in man; but every germ which causes disease in man does so by virtue of its chemie products, and if these be isolated and injected into the dog in sufficient quantity a poisonous effect will be produced. In the study of the bacteriology of the infectious diseases, the third and fourth of Koch's rules have not been complied with in many diseases, as has been stated, on account of the insusceptibility of the lower animals. The majority of investigators, meeting with this difficulty, have been inclined to rest content with the first two rules, and to conclude that when a given germ is constantly present in a given disease, and not found in other diseases, that it is the cause of the disease with which it is associated. Indeed, we find so good an authority as WELCH stating that the successful inoculation of animals is not necessary in order to prove the causal rela-

tionship of a germ to a disease. In 1889, VAUGHAN suggested that in those instances in which the third and fourth of Koch's rules cannot be complied with on account of the insusceptibility of the lower animals, it must be shown that the germ can produce chemie poisons which will induce in the lower animals in an acute form the characteristic symptoms of the disease, before the proof that the given germ is the cause of the disease be accepted as positive.

Heretofore, the science of bacteriology has been largely founded upon morphologic studies. Bacteriologists have given their time and attention to the discovery of bacterial forms in the diseased organism and to observations of characteristics in structure and growth of different species of bacterial life. We must now study the physiology and chemistry of the germs, and until this is done we must remain ignorant of the true cause of the disease, and so long as we remain ignorant of the cause it cannot be expected that we shall discover scientific and successful methods of treatment. Suppose that our knowledge of the yeast plant was limited to its form and method of growth; of how little practical importance this knowledge would be. That the yeast plant requires a saccharine soil before it can grow, that given such a soil it produces carbonic acid gas and alcohol, are the most important and practical facts which have been ascertained in its study. Likewise, the conditions under which pathogenic germs multiply and the products which they elaborate in their multiplication must be ascertained before their true relationship to disease can be understood.

In saying that the morphologic work upon which the science of bacteriology rests almost wholly is inadequate, we wish that it may be plainly understood that we are not offering any hostile criticism upon the great men who have done this work and who have formulated conclusions therefrom. The development of bacteriology has been in accordance with the natural law governing the growth of all the biological sciences. The study of form naturally and necessarily precedes the study of function. The ornithologist finds a new

species of bird. He first studies its shape and size, the color of its plumage, the form of its beak, the number and arrangement of the feathers of the tail and wing, the color of the eyes, etc. All this he can do with a single specimen, recognizing the fact, however, that variations more or less marked are likely to be found in other individuals. More time and wider opportunities of observation will be needed before he can tell where and when this bird is accustomed to build its nest, upon what insects, grains, and berries it feeds, with what other species of birds it lives in peace and with what it is at war. A much greater range of observation and study is necessary before the naturalist can tell how his newly discovered species would thrive if carried to a new climate, where it would be compelled to live upon unaccustomed food, to build its nest of strange material, and to encounter new foes.

We repeat that it is no discredit to the science nor to the men who have developed it to say that the study of bacteriology has hitherto been almost wholly morphologic. Without the morphologist the physiologist and the physiological chemist could not exist. The science having had for its support only morphologic studies, the deductions and formulated statements arrived at by its students have been reached in accordance with the knowledge obtained from this source. But now, it being admitted that the causal relation between a given germ and a certain disease is dependent upon the chemie products of the growth of the germ, the fundamental lines of work must be altered in order to correspond with this new knowledge.

"The study of the chemical factors in the causation of the infectious diseases opens up for us a field in which much work must be done. Let us attempt a statement of the nature of some of the researches that must be carried out along this line.

"In the first place, we must ascertain what germs are toxicogenic. This would necessitate a chemie study of all kinds of bacteria, both the pathogenic and the non-pathogenic.

Every fact ascertained in this investigation will not have its practical application in medicine, but will have its scientific value, and many will most probably be of more or less direct service to man.

"Secondly, it must be determined under what conditions these germs are toxicogenic. It is not at all probable that all those bacteria which are capable of producing poisons when grown on dead material outside of the body are also capable of multiplication and the production of the same substances when under the influence of the various secretions of the body. Some bacteria are destroyed by a normal gastric juice within a short time, while others are not. The conditions of life and growth are different when the infecting agent is introduced into the blood from what they are when infection occurs by the way of the alimentary canal. This is well recognized in the two forms of anthrax, one of which arises from inoculation through a wound and the other by way of the intestines. A preventive treatment which is efficient in one is of no service in the other. Then, again, we are to study those conditions of the blood and other fluids of the body which are especially unfavorable to the successful implantation or the continued existence of an infectious disease.

"Thirdly, the chemic properties and the physiologic action of these poisons will demand careful attention. Some are especially depressing in their action upon the heart, others seem to manifest their chief energy upon the central nervous system, while others still act like true gastro-intestinal irritants. In the study of the toxicologic effects of these bacterial poisons every method of investigation known in the most advanced physiologic work must be employed. The action of these agents on the heart, the brain, the spinal cord, etc., must be thoroughly studied."

The above quotations consist of paragraphs as they appeared in the second edition of this book. We cannot resist the temptation of calling attention to their verification. There is no department of bacteriology now receiving more attention than

that of the chemie nature and the toxicologic action of the bacterial products. Not only have the pathogenic germs been considered, but the great work of METSCHNIKOFF on the importance of saprophytic bacteria in the causation of Asiatic cholera illustrates the value of a knowledge of the physiology of all bacteria. It is now generally admitted that the summer diarrhœas of infancy are due not to a specific germ, but to any one of a number. Bacteria cannot be accurately classified, so far as their causal relation to disease is concerned (and this is the most important knowledge to be gained from them) until we know the nature of their chemie products, for it is by virtue of these that the germs have any causal relationship to disease. This knowledge is now being acquired more rapidly than it can be classified, and possibly more rapidly than its true significance can be comprehended.

CHAPTER VI.

THE BACTERIAL POISONS OF SOME OF THE INFECTIOUS DISEASES.

WE will now give our attention to the chemie poisons, both the ptomaïns and the toxins, of some of the infectious diseases, and in doing this we will illustrate and substantiate the statements made in the preceding chapter.

INFLUENCE OF BACTERIAL SUBSTANCES ON THE TEMPERATURE.—KREHL has studied the nature and origin of the fever-producing bacterial substances with reference to the following questions: (1) Do all bacteria induce fever? (2) Are all animals affected alike? (3) Is there any relation between the fever-producing properties of a given germ and its pathogenic effect upon the animal? The germs employed were *b. pyocyaneus*, *b. coli*, *b. anthracis*, *b. typhus abdominalis*, *b. diphtherie*, *b. cholerae*, *b. proteus*, *b. subtilis*, *b. prodigiosus*, and *b. Metschnikovi*. The animals experimented upon were cats, dogs, rabbits, guinea-pigs, pigeons, chickens, and a hedge-hog. Growths, two or three days old on agar-agar or potato, were removed with a sterilized metallic spatula, suspended in sterilized water and boiled. Bouillon cultures sterilized by heat were also employed.

In the pigeons, chickens, and the hedge-hog no elevation of temperature could be induced. In the pigeons, the temperature was generally lowered. Rabbits were found to be especially susceptible. However, bouillon cultures of the chicken cholera germ and of the *subtilis* did not act more energetically upon rabbits than uninoculated bouillon. Dogs were less easily affected; only five of the germs used caused an elevation of temperature in these animals. Different germs

acted differently upon guinea-pigs, but, as a rule, small doses elevated, while larger quantities depressed the temperature. There was found to be no constant relation between the fever-inducing and the pathogenic properties of the bacteria. Boiled diphtheria bacilli had practically no effect upon the temperature of guinea-pigs, rabbits, and dogs, while the *baecillus subtilis*, wholly devoid of pathogenic properties, markedly elevated the temperature of dogs.

In order to ascertain the nature of the fever-inducing substances, the following experiment was employed: Three grams of dried bacilli coli were suspended in one-half litre of water, heated for thirty minutes at 98°, filtered, and 5 c.c. of the clear filtrate injected subcutaneously in a rabbit caused high fever. The filtrate, with feebly acid reaction, was saturated with ammonium sulphate, the resulting precipitate was separated, freed from the salt and dissolved in sterilized water. This solution gave the reactions of the albumoses and contained 0.34 per cent. of proteid material. When this solution was injected into rabbits, it caused an elevation of temperature, but less marked than that induced by the original filtrate.

It was ascertained that certain substances of non-bacterial origin elevate the temperature when given subcutaneously. Physiologic salt solution, distilled water, and solution of grape-sugar were without effect. Sterilized egg albumin had only a slightly elevating effect, while that of sterilized milk was more noticeable. It was observed that it made a difference whether the same animal was used a second time or a new one treated each time. Repeating the experiment on the same animal seemed to increase the effect on the temperature. Certain enzymes, pepsin, rennet, diastase, invertin, and papain, boiled or unboiled, elevated the temperature of rabbits, guinea-pigs, and dogs. Pepton was inconstant in its action, the smallest doses affecting the temperature of guinea-pigs, while rabbits and dogs did not react. Bouillon markedly elevated the temperature of guinea-pigs. Five per cent. sterilized solutions of sodium nitrate, chlorate, iodid, and bromid caused fever in rabbits.

The temperature of tuberculous animals was found to be more easily affected than that of the healthy. Paralactic acid was found to have the same action as tuberculin, and on section of the tuberculous animals treated with this agent the diseased areas were observed to be hyperemic and to contain hemorrhagic spots.

The fact that Koch's tuberculin caused an elevation of temperature in consumption, while the same doses produced no visible effect upon the healthy, and the further fact that the tuberculin caused a local reaction in tuberculous areas, led to the general belief that a specific for tuberculosis had been discovered. Unfortunately, however, the action of the various constituents of the nutritive media in which the tubercle bacillus had been grown in the preparation of tuberculin had never been tested. When these tests were made it was ascertained that the claims of a specific action of tuberculin rested upon small support. Not only has it been shown that cultures of other microorganisms induce all the symptoms observed under the administration of tuberculin, but that certain proteids of non-bacterial origin act in the same manner. MATTHIES has contributed a valuable report on the effects of subcutaneous injections of certain albumoses on animals and especially upon tuberculous animals. The albumoses employed were obtained both by artificial digestion and by the action of superheated steam on albumins, and consisted of heteroalbumose, deutoalbumose, and athmidalbumose.

Doses of from three-tenths to one gram of deutoalbumose injected under the skin of healthy guinea-pigs induced a temperature as high as 41.3° , but without any more serious effect. On the other hand, doses of from one to five-tenths gram proved fatal to tuberculous pigs. Section showed recent inflammatory action in the tuberculous areas exactly identical with those caused by tuberculin. Smaller doses, from 0.01 to 0.02 gram, had no effect on the temperature of the healthy animals, but produced a marked febrile reaction in the tuberculous.

The other albumoses produced similar, but less marked, effects. Moreover, it was shown by beginning with small

doses that a certain degree of immunity to the effects could be established.

After experimenting on guinea-pigs and rabbits, MATTHES tested the action of deuteroalbumose on man. Subcutaneous injections of 0.07 gram caused marked febrile reactions in healthy men, and in three cases of lupus, in addition to the fever, a marked local reaction was observed.

The only difference between the effects of tuberculin and those of deuteroalbumose observed was that larger doses of the latter were required to cause a given effect. From this MATTHES concludes that tuberculin contains in addition to deuteroalbumose some true pepton, or that tuberculin consists of a mixture of digestive products. True pepton in doses of 5 and 20 milligrams induced violent reactions in two tuberculous guinea-pigs, and only the one receiving the smaller quantity recovered.

In conclusion, MATTHES advises that the inexpensive, easily prepared, and permanent deuteroalbumose be substituted for tuberculin in all cases in which the latter is employed.

CENTANNI prepares the bacterial fever-poison, which he names pyrotoxina bacterica, in the following manner: Cultures in liquid media that do not contain pepton and are several weeks old are employed. The culture is first kept for three hours at 60° and next boiled for the same length of time, the water of evaporation being replaced. Then the fluid is filtered through porcelain and evaporated to a syrup. Absolute alcohol is added. The precipitate formed is extracted with water, filtered, and the filtrate placed in a parchment dialyzer in a vessel of distilled water. The addition of a little chloroform or thymol prevents putrefaction. The dialysate of the first twenty-four hours is discarded on account of the large amount of salts and coloring matters which it contains; that of the second and third days is combined and evaporated to a small volume. This is treated with absolute alcohol, and the precipitate, purified by repeated resolution in water and reprecipitation with absolute alcohol, constitutes the pyrotoxina bacterica, which is at last separated from the

alcohol by decantation and dried in vacuo over sulphuric acid.

Pyrotoxina bacterica, prepared as stated above, is a grayish-white substance, that appears under the microscope in the form of fine granules. It is very hygroscopic and decomposes after short exposure to the air. It is soluble in water of neutral, feebly alkaline or feebly acid reaction, or when it contains neutral salts. It is also soluble to some extent in dilute alcohol, and a strength of more than 90 per cent. is necessary for its precipitation. It may remain under alcohol for many hours without losing its active properties. Glycerin also dissolves pyrotoxin and may be used for extracting it from bacterial cells. It is not soluble in ether or chloroform and is not altered by them. It is not an albuminoid body and may be obtained from bacteria grown in urine or in Nägeli's medium. It does not respond to the biuret, xanthoproteic, Millon or Adamkiewicz tests for proteids. It is not precipitated by acetic acid and potassium ferrocyanid. Therefore it is not a toxalbumin. It is not basic and therefore not a ptomain. It withstands boiling and is therefore not an enzyme. Moreover, its effects are in direct proportion to the amount administered. It is precipitated by tannic acid, lead acetate, mercuric chlorid, and phosphomolybdic acid, but not by gold or platinum chlorid.

Pyrotoxin induces the complex of symptoms included under the term fever. In the first place, it causes an elevation of temperature, the curve, beginning with a slight depression, rises to the acme, and then slowly falls to normal. The height and continuance of the fever are proportional to the quantity of the substance used. In the second place, it causes emaciation, which may continue as an incurable marasmus. After very large single injections the emaciation may continue for weeks after the disappearance of the fever and finally terminate in death. When very small doses are repeatedly given there may be no appreciable elevation of temperature, and yet the marasmus will follow. In the third place, the digestive organs are impaired, the appetite is lost, and diarrhoea sets

in. Lastly, other symptoms of the infectious fevers are not wanting. The frequency of the heart-beat and of the respiration is increased. The sensorium is benumbed and lack of co-ordination is observed. Prostration and indifference to surroundings mark the last hours.

Pyrotoxin is found in all kinds of bacteria examined, both the pathogenic and the non-pathogenic, and there seems to be no relation between the quantity of this substance contained in the bacterial cells and the pathogenic action of the living germ, inasmuch as some non-pathogenic organisms are found to yield the largest quantities of the poison.

Pyrotoxin is a constituent of the bacterial cell and not a split product formed by the analytic action of the bacterium on constituents of the medium in which it grows. For this reason it is more abundant in old cultures in which the cells are broken down and their contents dissolved, than in new cultures the cells of which are morphologically intact. This indicates that in the infectious diseases a number of bacteria must grow, reach maturity, and undergo dissolution before the existence of the disease is indicated by an elevation of temperature. The time necessary for these changes to be accomplished is the true period of incubation.

CENTANNI and BRUSCHETTINI report that the blood serum of animals that have been artificially rendered immune to the influenza bacillus neutralizes the effects of pyrotoxin, prepared from any germ, when employed either as a prophylactic or curative agent. This statement should be confirmed before general credence is given it. If it be true, an antipyretic applicable to all febrile diseases has been discovered.

MARAGLIANO has studied the temperature curves of some infectious diseases and arrived at certain conclusions which are of interest in this connection. The patients selected were those who had uncomplicated diseases, and no medicines which could influence the temperature were given. The number of patients with pneumonia studied in this way was 268. The crisis was reached in two on the second, in three on the third, in seven on the fourth, in thirty-five on the fifth, in

forty-one on the sixth day, altogether in eighty-eight before the seventh day. He concludes that when there is a single focus of infection or many foci developing simultaneously, and no new foci are formed, the fever never continues more than seven full days or 168 hours. When the fever is of longer continuance, it indicates that new foci of infection are developed or complications have arisen. When new foci are developed, before the fever due to the primary ones has disappeared, the fever continues, but that due to the new infection does not continue for seven days on account of the development of a condition of autoimmunization, and the period of fever arising from successively developed centres of infection grows shorter.

In lobar pneumonia, whatever germs may cause it, the temperature curve is the sum of the curves arising from successively developed centres of infection. The type of the curve is remittent and the remissions are the more marked the longer the fever continues. So long as fever continues, bacterial poisons are entering the circulation and danger is not past. As soon as the fever wholly disappears, systemic infection ceases and the danger is past. However, the lesion does not disappear until later, but the infecting bacteria are no longer active.

In pleurisy the continuance of the fever depends upon the nature of the infecting agent. However, in this disease, whatever the nature of the germ causing it, successive foci of infection are likely to develop, the secondary ones causing a fever less both in degree and in continuance than that due to the primary infection.

In so-called rheumatic polyarthritis, the number of joints involved, provided that all are involved at the same time, has no influence upon the length of time of the continuance of the fever.

In typhoid fever, the temperature curve is subject to great variations on account of the successive development of new foci and the effect of mixed infection. In making sections after death from this disease, the existence of multiple points

of infection showing differences in stage of development is frequently observed.

The curve of erysipelas is also quite variable. The fever of uncomplicated measles and scarlet fever does not continue longer than seven days.

The conclusions of interest to us, from the above-mentioned observations, may be stated as follows: The bacterial toxins cause the fever of the infectious diseases. At a given point of infection the number of germs which will develop is limited; and in the same tissue of one individual, during the course of a disease, the number developing at different points of inoculation is probably fairly constant, varying somewhat with the virulence of the germ and the number of germs which locate, as it were, at the different points. This is similar to the facts daily observed in our artificial cultures. Take two or any number of tubes of the same gelatin and inoculate them at the same time with the typhoid germ and keep the tubes under the same conditions, and the rate and limit of growth will be practically the same in all the tubes. In none of them is the growth unlimited. In none of them is all the nutrient material of the gelatin utilized before the growth stops. This can be demonstrated by making a second inoculation in the same tube parallel with the first and after the first has ceased to develop. There is a limit to the amount of wheat that can be produced from one bushel used as seed, and there is also a limit to the quantity of wheat which a given area will produce. Moreover, the wheat grows only where the seeds fall. Sowing the seed on one acre will not cause wheat to grow on an adjoining acre, but if the wheat grown on one acre be strewn over the adjoining acre, then a second crop will be produced here. This probably represents the conditions that obtain in the acute infectious diseases. The thermogenic bacterial products are cellular substances and the number of bacteria developed represent the quantity of these products elaborated.¹

¹ The statement of these conclusions is not as given by MARAGLIANO, but as interpreted by the writers of this book.

ANTHRAX. The definition of an infectious disease, as we have given it, is well illustrated by the facts which have been learned concerning the causation of anthrax, which has probably been more thoroughly studied than any other infectious disease. KAUSCH taught that this disease has its origin in paralysis of the nerves of respiration. As to the cause of this paralysis he gave us no information. DELAFOND thought that anthrax has its origin in the influence of the chemie composition of the soil affecting the food of animals and leading to abnormal nutrition. The investigations of GERLACH in 1845 demonstrated the contagious nature of the disease, which was emphasized by HEUSINGER in 1850 and accepted by VIRCHOW in 1855. However, as early as 1849, POLLENDER found numerous rod-like microorganisms in the blood of animals with the disease. This observation was confirmed by BRAUELL, who produced the disease in healthy animals by inoculations with matter taken from a pustule on a sick horse. Attempts were made to ridicule the idea that these germs might be the cause of the disease, and it was said that the bodies seen were only fine shreds of fibrin or blood crystals. Some claimed that the rod-like organisms reported were due to defects in the glass, while others claimed that the defects existed in the eye of the observer, and others still suggested that the defects might be found back of the eye and in the brain. But in 1863, DAVAINÉ showed that these little bodies must have some causal relation to the disease, inasmuch as his experiments proved that inoculation of healthy animals with the blood of those sick with anthrax produced the disease only when taken at a time when the blood contained these organisms. He also demonstrated beyond any question that these rod-like bodies are bacteria, capable of growth and multiplication. The conclusions of this investigator were combated by many; but PASTEUR, KOCH, BOLLINGER, DE BARY, and others, studied the morphology and life-history of these organisms, and then came the brilliant results of PASTEUR and KOCH in securing protection against inoculation anthrax by the vaccination of healthy animals with the modified germ

and subsequent inoculation with the virulent form. Now, the bacillus anthracis is known in every bacteriological laboratory, and by inoculation with it the disease is communicated at will to susceptible animals. But here the question arose How do these bacilli produce anthrax? and in answer to this question the various theories which we have mentioned were proposed.

The first successful attempt to study the chemie poisons of anthrax was made by HOFFA, who obtained from pure cultures of the bacillus small quantities of a ptomain, which, when injected under the skin of animals, produces the symptoms of the disease and death. This substance causes at first increased respiration and action of the heart, then the respirations become deep, slow, and irregular; the temperature falls below the normal; the pupils are dilated, and a bloody diarrhoea sets in. On section the heart is found contracted, the blood dark, and ecchymoses are observed on the pericardium and peritoneum. HOFFA named his poison anthracin. Recently HOFFA has isolated this poison from the bodies of animals dead from anthrax.

It has been said that HOFFA's work was the first successful attempt to study the chemie poisons of anthrax. However, his results cannot be considered altogether satisfactory. The small amount of the basic substance which he obtained rendered it highly probable that in the case of a germ so virulent as that of anthrax there must be other chemie poisons produced. This supposition has been confirmed by the labors of HANKIN, who, in 1889, while at work in Koch's laboratory, prepared from cultures of the bacillus anthracis an albumose which, when employed in comparatively large amount, proved fatal to animals, but when used in very small quantity gave immunity against subsequent inoculations with the living germ. Unfortunately, HANKIN does not mention the symptoms induced by toxic doses of this substance. Whether or not the albumose of HANKIN contains in *statu nascendi* the base of HOFFA, and owes its poisonous properties to the same, has not been determined.

BRIEGER and FRAENKEL obtained a so-called toxalbumin of anthrax from animals in which the disease had been induced by inoculation with the bacillus. The liver, spleen, lungs, and kidneys of these animals were finely divided and rubbed up with water. After this had stood in a refrigerator for twelve hours it was passed through a Chamberland filter and the proteid precipitated from the filtrate with absolute alcohol.

MARTIN, by growing the anthrax bacillus for from ten to fifteen days in an alkaline albuminate from blood serum and filtration through porcelain, obtains the following metabolic products:

1. Protoalbumose and deutoalbumose and a trace of pepton. All of these react chemically like similar substances prepared by peptic digestion.
2. An alkaloid.
3. Small quantities of leucin and tyrosin.

The most characteristic property of the albumoses is that their solutions are strongly alkaline, and the alkalinity is not removed by treatment with alcohol, benzol, chloroform, or ether, or by dialysis.

The alkaloid is soluble in water, alcohol, and amyllic alcohol; insoluble in chloroform, ether, and benzol. Its solutions are strongly alkaline and the alkaloid forms crystalline salts with acids. It is precipitated by the general alkaloidal reagents, with the exception of potassio-mercuric iodid. It is somewhat volatile and loses its poisonous properties on exposure to the air.

The mixed albumoses are poisons only in considerable doses, 0.3 gram being required to kill a mouse of 22 grams weight when injected subcutaneously. Smaller doses cause a local oedema and a somnolent condition, from which the animal recovers. The larger doses produce a more extensive oedema and the somnolence deepens into coma, terminating in death. In some cases the spleen is enlarged. The absence of germs was demonstrated by plate cultures. The alkaloid causes similar symptoms. It is, however, more poisonous and acts

more rapidly than the albumoses. The animal is affected immediately after the injection, and the gradually increasing coma terminates in death. The alkaloid also produces cedema, and in many cases thrombosis of the small veins. Extravasation into the peritoneal cavity is occasionally seen, and the spleen is ordinarily enlarged and filled with blood. The fatal dose for a mouse is from 0.1 to 0.15 gram, death resulting within three hours.

This alkaloid does not appear to be identical in its action with the anthracin of HOFFA.

Numerous attempts have been made recently to isolate the anthrax toxin. None of these have been wholly satisfactory. The best, as well as the most recent (1895), work done in this direction is that of MARMIER. The culture medium employed had the following composition :

Water	1000	grams.
Pepton	40	"
Sodium chlorid	15	"
Sodium phosphate	0.5	gram.
Potassium phosphate	0.2	"
Glycerin	10	"

The pepton was obtained from the commercial preparation by precipitation of the other proteids with ammonium sulphate. This salt was removed by dialysis or by precipitation with barium hydrate. In the above-given menstruum the anthrax bacillus, especially the sporeless variety, grew abundantly. The toxin was obtained from the culture by precipitation with ammonium sulphate also. The dried toxin is soluble in water and in 1 per cent. solution of phenol; insoluble in chloroform, ether, and absolute alcohol. It is said not to give any of the reactions of the albuminoids, propeptons, peptons, or alkaloids, but since there is no mention of the reactions tested, and since precipitation with ammonium sulphate is an albumin and propepton reaction, this statement must be considered as somewhat indefinite and possibly as misleading. There is no proof that the toxin was pure. In fact, the report makes the reader quite certain that it was not by any means chemically pure. The author was surprised

in studying the poisonous effects of this toxin to find that while twenty milligrams killed some rabbits, others resisted seventy milligrams; but it does not seem that he once thought that this might be due to the fact that the mass of his product was inert, and that these differences in effects were due to the unequal distribution of the active agent. However, we will give the author's conclusions as he states them :

1. A specific toxin may be extracted from glycerin-pepton cultures of the anthrax bacillus.

2. This toxin does not give the reactions of albuminoid substances. It does not change starch, sugar, or glycogen.

3. Animals (chickens, frogs, fish) that are immune to the anthrax bacillus are also indifferent to the toxin. Similar results were observed in rabbits artificially immunized with attenuated cultures

4. This toxin is attenuated, but not destroyed, by boiling at 110° , thus differing from the venom of serpents, the toxins of diphtheria and tetanus, and the enzymes.

(It is more than likely that this result also was due to the mixture of the toxin with an inert body.)

5. On the contrary, like the other bacterial toxins, it loses its action on animals after being brought in contact with the alkaline hypochlorites. Prolonged insolation in the presence of air leads to the same result.

6. By employing carefully graduated doses of this toxin it is possible to immunize animals to the anthrax bacillus, in the same way as other specific toxins give immunity to the corresponding diseases.

7. Anthrax cultures in other fluids, such as blood serum and bouillon from the flesh of the horse, ox, or calf, do not contain the toxin in appreciable quantities.

8. On the contrary, one may extract a toxin from anthrax cultures on nutritive gelatin by macerating the microbes in alcoholic water (20 per cent. alcohol).

9. The toxin is contained within the bacterial cells, and in order to obtain it in a culture there must be opportunity for it to diffuse from the cells.

Whether the toxin as obtained by MARMIER was chemically pure or not, there can be no doubt that he was dealing with the specific poison of anthrax. The symptoms induced by this toxin are identical with those observed in the same animals after infection with the anthrax bacillus.

ASIATIC CHOLERA. There are good reasons, apart from experimental evidence, for believing that the comma bacillus of KOCII produces its ill effects by the elaboration of chemie poisons. This germ is not a blood parasite. It grows only in the intestine, and the symptoms of the disease and death must result from the absorption of its poisonous products. In confirmation of this statement experiment has shown that this is one of the most active, chemically, of all known pathogenic germs.

In the first place, BITTER has shown that the comma bacillus produces in meat-pepton cultures a peptonizing ferment, which remains active after the organism has been destroyed. Like similar chemie ferments, it converts an indefinite amount of coagulated albumin into pepton. It is more active in alkaline than in acid solutions, thus resembling pancreatin more than pepsin. This resemblance to pancreatin is further demonstrated by the fact that its activity is increased by the presence of certain chemicals, such as sodium carbonate and sodium salicylate. That a diastatic ferment is also produced by the growth of the bacillus was indicated in the experiments of BITTER by the development of an acid in nutrient solutions containing starch-paste. However, all attempts to isolate the diastatic ferment were unsuccessful. A temperature of 60° destroys or greatly decreases the activity of ptyalin, and this seems to be true also of the diastatic ferment produced by the comma bacillus; but the formation of an acid from the starch presupposes that the starch is first converted into a soluble form.

FERMI has succeeded in isolating the peptonizing ferment of the cholera germ in the following manner: 65 per cent. alcohol added to gelatin which has been liquefied by the bacil-

his precipitates the proteid, but not the ferment. After twenty-four hours the precipitate is removed by filtration and the ferment precipitated from the filtrate by the addition of absolute alcohol. After being collected on a filter and dried the ferment is dissolved in an aqueous solution of thymol and its peptonizing properties demonstrated on gelatin tubes.

RIETSCII believes that the destructive changes observed in the intestines in cholera are due to the action of the peptonizing ferment.

CANTANI injected sterilized cultures of the comma bacillus into the peritoneal cavities of small dogs and observed after from one-quarter to one-half hour the following symptoms: Great weakness, tremor of the muscles, drooping of the head, prostration, convulsive contractions of the posterior extremities, repeated vomiting, and cold head and extremities. After two hours these symptoms began to abate, and after twenty-four hours recovery seemed complete. Control experiments with the same amounts of uninfected beef-teen were made with negative results. The cultures used were three days old when sterilized. Older cultures seemed less poisonous and a high or prolonged heat in sterilization decreased the toxicity of the fluid. From these facts CANTANI concluded that the poisonous principle is volatile, but the effect of high or prolonged heat in diminishing the toxicity was more probably due to its destructive effect on the toxins.

CANTANI also observed that the blood of those sick with cholera is acid: this has been confirmed by STRAUSS by the examination of the blood directly after death; and AUREND found lactic acid in the strongly acid urine of a cholera patient.

NICATI and RIETSCII produced fatal effects in dogs by injecting cultures, from which all germs had been removed by filtration, into the bloodvessels. Later, the same investigators obtained from old bouillon cultures containing pepton a poisonous base. ERMENGEX also showed that cultures after filtration through a Chamberland filter are poisonous.

KLEBS has attempted to answer experimentally the ques-

tion, In what way does the cholera germ prove harmful? Cultures of the bacillus in fish preparations were acidified, filtered, the filtrate evaporated on the water-bath, the residue taken up with alcohol and precipitated with platinum chlorid. The platinum was removed with hydrogen sulphid, and the crystalline residue obtained on evaporation was dissolved in water and injected intravenously into rabbits. Muscular contractions were induced. Death followed in one animal, which, in addition to the above treatment, received an injection of a non-sterilized culture. In this case there was observed an extensive calcification of the epithelium of the uriniferous tubules. KLEBS believes this change in the kidney to be induced by the chemie poison, and from this standpoint he explains the symptoms of cholera as follows: The cyanosis is a consequence of arterial contraction, the first effect of the poison. The muscular contractions also result from the action of the poison. The serous exudate into the intestines follows upon epithelial necrosis. Anuria and the subsequent symptoms appear when the formation and absorption of the poison become greatest.

HUEPPE states that the severe symptoms of cholera can be explained only on the supposition that the bacilli produce a chemie poison, and that this poison resembles muscarin in its action.

VILLIERS isolated by the Stas-Otto method from two bodies dead from cholera a poisonous base which was liquid, pungent to the taste, and possessed the odor of hawthorn. It was strongly alkaline, and gave precipitates with the general alkaloidal reagents. From one to two milligrams of this substance, injected into frogs, caused decreased activity of the heart, violent trembling, and death. The heart was found in diastole and full of blood, and the brain slightly congested. However, the presence of this substance in the bodies of persons who have died of cholera does not prove that its production is due to the cholera bacillus.

POUCHET extracted from cholera stools, with chloroform, an oily base belonging to the pyridin series. It readily reduces ferric as well as gold and platinum salts, and forms an easily

decomposable hydrochlorid. It is a violent poison, irritating the stomach, and retarding the action of the heart. Subsequently, he obtained an apparently identical substance from cultures of Koch's comma bacillus.

In 1887, BRIEGER made a report of his studies on the chemistry of the cholera bacillus. He used pure cultures on beef-broth (*fleischbrei*), which was rendered alkaline by the addition of a 3 per cent. soda solution. These were kept at from 37° to 38°. After twenty-four hours, eadaverin was found to be present. Older cultures furnished very small quantities of putrescin, but cultures on blood serum yielded much larger amounts of this base. While eadaverin and putrescin cannot be said to be poisonous, they do cause necrosis of tissue into which they are injected, and their formation by the cholera bacillus may account for the necrotic tissue in the intestine in the disease. The lecithin of the beef-broth was slowly acted upon by the germs, but with age the amount of cholin increased, reaching its maximum during the fourth week.

Creatin proved still more resistant to the action of the germs; but, after six weeks, a considerable quantity of creatinin was isolated, and a similar amount of methyl-guanidin. The latter is very poisonous, causing muscular tremors and dyspnoea. The presence of methyl-guanidin indicates that the comma bacillus acts as an oxidizing agent, since creatin yields methyl-guanidin only by oxidation.

BRIEGER succeeded in finding, in addition to the above-mentioned ptomaines, which are common products of putrefaction, two poisons which he considered as specific products of the comma bacillus. One of these, found in the mercuric chlorid precipitate, is a diamine, resembling trimethylenediamine. It produced muscular tremor and heavy cramps. In the mercury filtrate was found another poison, which, in mice, produced a lethargic condition; the respiration and heart's action became slow, and the temperature sank so that the animal felt cold. Sometimes there was bloody diarrhoea.

It is very probable now (1895) that many, possibly all, of these basic substances found by BRIEGER in cultures of the

cholera germ are artificial products formed during the process of extraction. Whether this be true or not, it is quite certain that they are not prominent factors in the production of the symptoms or in causing death. For the work of Roos on cholera ptomaïns, see section on leucomaïns of the urine.

BRIEGER and FRAENKEL found that the insoluble proteid which they obtained from cultures of the cholera bacillus, when suspended in water and injected subcutaneously in guinea-pigs, caused death after from two to three days. Section showed inflammatory swelling and redness of the subcutaneous tissue, extending into the muscles for some distance about the point of injection, but no necrosis. There was no change in the intestines and no effusion into the peritoneum. In some instances there was evidence of beginning fatty degeneration of the liver. Upon rabbits this substance, even in large doses, was without effect.

In endeavoring to obtain immunity in guinea-pigs against cholera, GAMALEIA employs cultures which have been sterilized at 120°. Subcutaneous injections of these cause transient œdema, and the animals soon recover. The high temperature destroys not only the bacillus, but renders inert certain "ferment-like" products. However, if the cultures be sterilized at 60°, large doses (10 c.c. per kilogram, body weight) cause death, injected intravenously in rabbits, and a less amount produces marked symptoms. The animals refuse food, and a diarrhœa, which may continue for hours, appears. Often there are cloudiness of the cornea and retention of urine, which is albuminous. The animals recover very slowly. In this connection BOUCHARD remarks that in 1884 he obtained by the intravenous injection of the urine of a cholera patient in rabbits muscular tremor, cyanosis, albuminuria, and diarrhœa, but that he has never succeeded in inducing these symptoms with the cholera vibrio.

PETRI finds that the comma bacillus produces in solutions of pepton large amounts of tyrosin and leucin, a small quantity of indol, fatty acids, poisonous bases, and a poisonous proteid. The proteid resembles pepton in its behavior toward heat

and chemical reagents, and is designated by PETRI as "toxopepton." It is not precipitated by heat or concentrated nitric acid, nor by potassium ferrocyanid and acetic acid, nor by ammonium sulphate added to saturation. With sodium phosphotungstate it gives a precipitate which clears up on the application of heat. The precipitate with tannic acid is insoluble in an excess of the precipitant. It gives the biuret reaction perfectly, but responds to Millon's test but feebly.

In quantities of 0.36 of a gram per kilogram, and more, it is fatal to guinea-pigs within eighteen hours. It produces muscular tremor and paralysis. Post-mortem examination shows an effusion into the peritoneal cavity, marked injection of the bloodvessels of the intestines, and isolated hemorrhagic spots.

This proteid is not rendered inert by a temperature of 100°. PETRI does not claim that he has obtained a chemically pure body, but supposes that it is contaminated with more or less unchanged pepton.

This substance contained the toxin of cholera, but the greater part of it consisted of proteid bodies.

SCHOLL has studied the chemical products of the cholera bacillus when grown under anaerobic conditions. Fresh eggs were sterilized and inoculated in the usual way. The eggs, after being kept for eighteen days at 36°, were opened. The contents smelled intensely of hydrogen sulphid, but not of amines. The albumin was completely fluid, while the yolk was more solid and of a dark color.

Five c.c. of the fluid contents were injected into the abdomen of a guinea-pig. Soon the posterior extremities were paralyzed, and after ten minutes the paralysis became general, the animal lying on the side. After five minutes more convulsive movements of the extremities began, and forty minutes after the injection the animal was dead. Section showed the vessels of the small intestine and stomach highly injected, a colorless effusion in the peritoneal cavity, and the heart in diastole.

The albuminous content of the egg was poured into ten times its volume of absolute alcohol. The precipitate was

collected and washed with alcohol until a colorless filtrate was obtained. The precipitate was then digested for fifteen minutes with 200 c.c. of water and filtered. Eight c.c. of the filtrate were injected into the abdomen of a guinea-pig. Paralysis resulted immediately, and within one and one-fourth minutes the animal was dead. Section showed marked injection of the vessels of the small intestine, a bloody transudate in the peritoneal cavity, and the heart in diastole.

The impure toxin was rendered inert by a temperature of 100° ; it was not altered by short exposure to 75° , but attempts to evaporate the solution at 40° in vacuo over calcium chlorid destroyed the poisonous properties. The proteid was finally precipitated from its aqueous solution by a mixture of alcohol and ether. It was washed with ether and the ether allowed to evaporate spontaneously. A small bit of this proteid proved fatal to guinea-pigs, and the same post-mortem changes were found as given above. SCHOLL classes this proteid among the peptons. It is not precipitated by heat or concentrated nitric acid, singly or combined, nor by ammonium sulphate. It gives the xanthoproteid and biuret reactions. SCHOLL regards this as a true poison of cholera, and points out its difference from the proteid of BRIEGER and FRAENKEL and that of PETRI.

GRIGORIEW has studied the action of the following vibrios on the contents of eggs: *v. cholerae*, *v. Metschnikovi*, *v. Finkler and Prior*, *v. Deneke*, and *v. aquatilis*. Of these germs only the first two are pathogenic to animals, and only these were found to elaborate powerful poisons in the eggs. The toxigenic properties of the *v. Metschnikovi* were found to be more marked than those of the *v. cholerae*.

BOXNORF finds that the vibrio *Danubicus*, *v. Berolinensis*, and *v. Dunbar* produce similar changes in the contents of eggs, although the poisons formed by the different vibrios vary in virulence. Moreover, with these poisons he was able to render animals immune to living cultures of the cholera vibrio.

HIERRE holds that the cholera poison results from the analytic or ferment action of the germs on the proteids in

which it grows, and that the proteids of the bacterial cells are not poisonous. With the classification which we have made in the first chapter of this book, HUEPPE would place the cholera-poison among our bacterial proteids and the immunizing substances among the bacterial cellular proteids. He claims that these substances can be separated in the following manner: Rice-water stools from cholera patients are treated with absolute alcohol. Both the toxin and the immunizing substance are precipitated. By collecting this precipitate and extracting it with sterilized water or physiologic salt solution, the toxins only are dissolved. The belief that a separation of these two classes of proteids can be made in this way rests upon the following assumptions, neither of which can be said to be demonstrated facts: (1) The poison and the immunizing body are not one and the same thing; (2) the cellular proteids are not soluble in water or salt solution.

HUEPPE claims, furthermore, that in a given case of cholera the toxin may be formed most abundantly and the immunizing substance only in small amount; in such a case the symptoms of the disease would be violent, and should recovery result, the immunity to subsequent infection would be slight. With the conditions reversed the disease might be slight and the immunity established great.

BUJWID found that on the addition of from five to ten per cent. of hydrochloric acid to bouillon cultures of the cholera bacillus there was developed after a few minutes a rose-violet coloration which increased during the next half-hour and in a bright light showed a brownish shade. The coloration is more marked if the culture is kept at about 37°. In impure cultures this reaction does not occur. The FINKLER-PRIOR bacillus cultures give after a longer time a similar, but more of a brownish coloration. Cultures of many other bacilli were tried and failed to give this reaction.¹

BRIEGER found that this color is due to an indol derivative.

¹ POEHL deserves the credit of being the first to call attention to this reaction, though his work was evidently unknown to Bujwid at the time when the latter published his report.

In cholera cultures on albumins he obtained indol by distillation with acetic acid.

BUJWID has made a further contribution to our knowledge of the "cholera-reaction." His conclusions are as follows:

(1) Five to ten per cent. of hydrochloric acid added to cholera cultures produces a rose-violet coloration, which is characteristic of the comma bacillus.

(2) No other bacterium gives the same coloration under the same conditions.

(3) The coloration appears in such cultures which are from ten to twelve hours old, so that this test can be used for diagnostic purposes, and will give results before they can be obtained by plate cultures.

(4) Impure cultures do not give this reaction.

DUNHAM finds the best medium for the "cholera-reaction" to be a one per cent. alkaline pepton solution with one-half per cent. of common salt. BUJWID prefers a two per cent. feebly alkaline pepton solution with salt. JADASSOHN finds that gelatin cultures give the reaction both before and after the liquefaction of the gelatin. The undissolved gelatin, after the addition of hydrochloric or sulphuric acid, becomes rose-violet.

COHEN claims that cultures of other bacilli give a similar coloration, but BUJWID explains that the results obtained by COHEN were due to the use of impure acids, which contained nitrous acid. SALKOWSKI agrees with BUJWID, and states that, when acids wholly free from nitrous acid are used, the reaction is characteristic of the comma bacillus. He explains the reaction by supposing that the germ produces nitrous acid, which exists in the culture as a nitrite. On the addition of an acid the nitrous acid is set free, and acting upon the indol, which is also present, gives the coloration. After all, Cohen was right; many germs give the indol reaction.

Cultures of the germ newly taken from the stools or intestinal contents do not give the indol reaction as constantly as those of germs being grown on artificial media. (HAMMURIL.)

From a very exhaustive research on the importance of this test PETRI comes to the following conclusions:

(1) Seven pure cultures of the cholera germ from as many sources gave the reaction with equal distinctness.

(2) Of one hundred other bacteria tested in the same way twenty gave a red coloration. In nineteen of these the coloration is due to the nitroso-indol reaction of Baeyer. The twentieth (anthrax) gave a color which is not due to indol.

(3) In case of the cholera germ, and the others as well, the reaction is due to the reducing action of the bacteria on nitrates. The reaction is most marked at blood-temperature and with the cholera bacillus; it is least distinct with the bacilli of FINKLER and MILLER.

(4) None of these bacteria convert ammonia into nitrite.

(5) The simple addition of sulphuric acid is sufficient to give the test, which, however, is most marked when the nutritive solution contains 0.01 per cent. nitrate.

(6) The reaction is most marked if the sulphuric acid be added after the addition of a very dilute nitrite solution.

SCHUCHARDT calls attention to the fact that VIRCHOW observed a red coloration on the addition of nitric acid to filtered cholera stools in 1846. GRIESINGER, in 1885, also made mention of the production of a red coloration in rice-water stools on the addition of nitric acid.

A "cholera-blue" has also been observed by BRIEGER in cultures in meat extract containing pepton and gelatin. This substance, which is yellow by reflected, and blue by transmitted light, is developed by the addition of concentrated sulphuric acid to the culture. It may be separated from the "cholera-red" as follows: Treat the culture with sulphuric acid, then render alkaline with sodium hydrate, and extract with ether. Evaporate the ether, and remove the "cholera-red" with benzol, then again dissolve the "cholera-blue" in ether. The characteristic absorption-bands for this coloring-matter begin in the first third of the spectrum, between E and F, and darken all of the zone lying beyond.

WINTER and LESAGE treat a bouillon culture of the cholera

germ with sulphuric acid, dissolve the precipitate in an alkaline medium, reprecipitate with acid, and redissolve in ether, which on evaporation leaves oily drops, and these, on cooling, form a yellow mass of the appearance of a fat. This substance is insoluble in water and acids soluble in alkalies and ether. It melts at 50° , and does not lose its virulence on being boiled with alcohol rendered feebly alkaline. The virulence of a culture and the amount of this substance contained therein are in direct proportion to each other.

Small doses of this substance (1 milligram to 100 grams of body-weight of the animal) in feebly alkaline solution introduced into the stomachs of guinea-pigs cause, as a rule, within from four to six hours, a chill, and death after twenty-four hours. With larger doses the temperature falls after from one-half to one hour, and death results within from twelve to twenty hours. Smaller doses cause a less marked reaction and the animal recovers within twenty-four hours. If killed within this time, the animal shows a choleraic condition. Rabbits succumb only after repeated subcutaneous injections. The substance can be extracted from the muscles, liver, kidneys, and urine of the poisoned animals. It can also be obtained from cultures of a cholera infantum germ. The fact that this poison belongs neither to the ptomaines nor albumins is of interest.

It is almost certain that this substance obtained by WINTER and LESAGE is an artificial product.

CUNNINGHAM describes ten species of the comma bacillus, one of which does not liquefy gelatin, and fails to respond to the cholera reaction. He also states that there are cases of undoubted cholera in Calcutta in which the comma bacillus is wholly wanting.

Much having been said about the identity or non-identity of Koch's bacillus and the Massana cholera bacillus, RONTALER has compared their chemie products. He finds that both germs produce the same optically inactive lactic acid, and that the only difference he could find is that the Massana germ produces larger amounts of indol, skatol, and fatty acids.

However, he places no significance on this, because the Koch germ produces these substances in variable quantities.

TETANUS.—In 1884, NICOLAÏER, by inoculating 140 animals with earth taken from different places, produced symptoms of tetanus in 69 of them. In the pus which formed at the point of inoculation he found micrococci and bacilli. Among the latter was one which was somewhat longer and slightly thicker than the bacillus of mouse septicæmia. In the subcutaneous cellular tissue he found this bacillus alone, but could not detect it in the blood, muscles, or nerves. Heating the soil for an hour rendered the inoculations with it harmless. In cultures, NICOLAÏER was unable to separate this bacillus from other germs, but inoculations with mixed cultures produced tetanus. In the same year, CARLE and RATONE induced tetanus in lower animals by inoculations with matter taken from a pustule on a man just dead from tetanus. In 1886, ROSENBACH made successful inoculations on animals with matter taken from a man who had died from tetanus consequent upon gangrene from frozen feet. With bits of skin taken from near the line of demarcation he inoculated two guinea-pigs on the thigh; tetanic symptoms set in within twelve hours, and one animal died within eighteen, and the other within twenty-four hours. The symptoms correspond exactly with those observed in the "earth tetanus" of NICOLAÏER, and the same bacillus was found. With mixed cultures of this, ROSENBACH was also able to cause death by tetanus in animals. BEUMER had under observation a man who died from lockjaw following the sticking of a splinter of wood under his finger-nail. Inoculations of mice and rabbits with some of the dirt found on the wood led to tetanus. The same observer saw a boy die from this disease following an injury to the foot from a sharp piece of stone. White mice inoculated with matter from the wound, and those inoculated with dirt taken from the boy's playground, died of tetanus. The bacillus of NICOLAÏER was again detected. GIORDANO reports the case of a man who fell and sustained a complicated

fracture of the arm. He remained on the ground for some hours, and when assistance came the muscles and skin were found torn and the wounds filled with dirt. On the fifth day he showed symptoms of tetanus, from which he died on the eighth day. Inoculations and examinations for the bacillus were again successful. FERRARI also made successful inoculations with the blood taken during life from a woman with tetanus after an ovariectomy. HOCKSINGER has confirmed the above-mentioned observations by carefully conducted experiments, the material for which was furnished by a case of tetanus arising from a very slight injury to the hand, the wound being filled with dirt. SHAKESPEARE has succeeded in inducing tetanus in rabbits by inoculating them with matter taken from the medulla of a horse and of a mule, both of which had died from traumatic tetanus. These uniform observations leave no room to doubt that tetanus is often, at least, due to a germ which exists in many places in the soil, and that the disease is transmissible by inoculation.

BONOME observed nine cases of tetanus among seventy persons injured by the falling of a church from the earthquake at Bajardo. The bacillus of NICOLAIER was detected in the wounds, and animals inoculated with the lime-dust of the fallen building died of tetanus. Of many persons injured by the falling of another church at the same time, none had tetanus, and animals inoculated with the lime from this church suffered no inconvenience.

The same experimenter found the bacillus in the wound of a sheep that died from tetanus after castration.

BEUMER found the tetanus bacillus in the sloughing tissue of the umbilical cord of a child which was taken ill on the sixth day after birth, and died four days later from tetanus. From this he concludes that tetanus neonatorum and "earth tetanus" are identical, and advises that the cord should be dressed antiseptically.

KITASATO succeeded in isolating the bacillus of Nicolaier by growing the mixed cultures, from the pus of a wound on a man who died from tetanus, and then heating the same at a

high temperature (80°) to destroy the common bacteria. The spores of the tetanus bacillus resist this heating and are subsequently developed under hydrogen. The bacillus grows only in the absence of air, and not in carbonic acid. It develops on agar-agar, blood serum, and gelatin, the last of which it gradually liquefies with the formation of gas. The growth is more vigorous when the nutritive medium contains from 1.5 to 2 per cent. of grape-sugar.

In 1888 BELFANTI and PESCAROLO found in the pus of a wound, which was followed by tetanus, a bacillus which they believed to differ morphologically from that of Nicolaier and Rosenbach, and which in pure cultures induced tetanus in animals. The number of animals experimented upon was great, and included mice, guinea-pigs, frogs, rabbits, pigeons, geese, sparrows, a chicken, and a dog. The pigeons, chicken, geese, and frogs proved immune. After subcutaneous injections a bloody œdema appeared at the place of inoculation and pus formed in small quantity. Paralysis first appeared and was followed by convulsions and opisthotonos. Later studies lead BELFANTI and PESCAROLO to conclude that their bacillus is really that of Nicolaier, but differing somewhat from that of Kitasato. KITASATO states positively that the germ which he has isolated is absolutely anaerobic, while the Italians find that theirs will not only grow aerobically, but when so grown will induce a classical tetanus. It is probable that they had in their hands a mixed culture containing the tetanus bacillus, for Novy has shown that anaerobic bacteria mixed with such aerobic germs as *micrococcus prodigiosus* or *Proteus vulgaris*, will grow in liquid media under aerobic conditions.

LAMPIASI found in the blood from various organs of a man who died from so-called spontaneous tetanus, and in two cases of tetanus in mules, a spore-forming bacillus, which in pure cultures induced tetanus in animals. This bacillus is wholly different morphologically from that of Nicolaier.

WIDENMANN reports a very interesting case of a boy who fell from a wall and wounded his face on a piece of vine-stake in the earth. The boy died of tetanus, and the splinters extracted from the face and the earth about the stake were

examined. The splinter was introduced under the skin of a mouse, which died thirty hours later of tetanus. In the pus formed about the splinter innumerable microorganisms, among which a micrococcus and a short, thick bacillus abounded, were found, but in none of the many animals experimented upon could the bacillus of Nicolaier be detected. In animals inoculated with the earth, however, the Nicolaier germ was found. WIDENMANN concludes that the so-called tetanus bacillus is found in most cases on account of its very wide distribution in the soil and not as a result of its causal relation to the disease. It is needless to remark that this conclusion is absurd.

FLÜGGE has produced tetanus in animals without being able to find the bacillus of Nicolaier; and WYSSOKOWITSCH has examined an earth which did not induce tetanus, but which caused suppuration, and in the pus the Nicolaier bacillus was found to be abundant. With the pus obtained from three cases of tetanus neonatorum due to omphalitis KISCHENSKY induced tetanus in animals. The pus contained pyogenic micrococci and a short bacillus, but the germ of Nicolaier could not be detected.

It must be understood that failure to detect the tetanus bacillus does not prove its absence. Especially is this true of the researches made five or more years ago. Recent evidence points to the fact that, although there may be slight morphologic distinctions in the bacilli of different localities, the tetanus germ is widely distributed and is possessed of characteristics sufficiently well marked to lead to its recognition.

BRUEGER has obtained in the mixed cultures of the germ of Nicolaier and Rosenbach four poisonous substances. The first, tetanin, which rapidly decomposes in acid solutions, but is stable in alkaline solutions, produces tetanus in mice when injected in quantities of only a few milligrams. The second, tetanotoxin, produces first tremor, then paralysis, followed by severe convulsions. The third, to which no name has been given, causes tetanus accompanied by free flow of the saliva and tears. The fourth, spasmotoxin, induces heavy clonic and tonic convulsions.

BRIEGER has also isolated tetanin from the amputated arm of a man with tetanus, thus showing that this chemie poison is formed in the body as well as in the artificial cultures.

More recent researches lead us to attach but little importance to the crystalline bodies discovered by BRIEGER.

BRIEGER and FRAENKEL obtained a "toxalbumin" from a culture of Kitasato's germ in bouillon containing grape-sugar. This substance is soluble in water, and when injected in small amounts subcutaneously in guinea-pigs tetanus appears in about four days, and soon terminates fatally.

Later, BRIEGER and COHN prepared tetanus poison from cultures of the bacillus in veal broth containing one per cent. of pepton and one-half per cent. of common salt. These cultures were rendered germ-free by filtration through porcelain, and treated with ammonium sulphate to supersaturation. This throws the poison out of solution and it floats on the surface, from which it is removed by a platinum spatula. This crude poison, when dried in vacuo, is found to contain 6.5 per cent. of ammonium sulphate. Of the filtered culture 0.00005 c.c. suffices to kill mice. From one liter of the culture one gram of the dry substance was obtained, and of this 0.0000001 gram killed a mouse with the typical symptoms of tetanus. This crude product contains, besides the poison, albumins, pepton, amido-acids, volatile substances, and ammonium sulphate, with other salts. The albumin was removed by precipitation with basic lead acetate. The pepton, amido-acids and salts were removed by dialysis, and finally evaporation in vacuo at 20° to 22° removed the volatile substances. The toxin, thus obtained, is yellow, flaky, readily soluble in water, odorless, and similar in taste to gum Arabic. It turns polarized light slightly to the left. It fails to give the Millon and xanthoproteic reactions, but does give with copper sulphate and caustic potash a faint, violet coloration, not identical with the rose of the biuret reaction. With the exception of ammonium sulphate, the metallic salts, as sodium chlorid and sulphate, magnesium sulphate, potassium nitrate, mercuric chlorid, and potassium ferrocyanid with acetic acid, fail to pre-

precipitate the purified poison. Moreover, calcium phosphate which ROUX and YERSIN used for carrying down the diphtheria poison, also magnesium carbonate and aluminum hydrate, do not throw the tetanus poison out of solution. The poison contains no phosphorus and only unweighable traces of sulphur. From these observations, BRIEGER and COHN conclude, contrary to the former belief of BRIEGER and FRAENKEL, that the tetanus poison is no true proteid.

Of the best preparation obtained by these investigators 0.00000005 gram killed a mouse of 15 grams weight. The authors figure from this that the fatal dose for a man of 70 kilos. would be 0.00023 gram, or 0.23 milligram, and 0.04 milligram would induce symptoms of tetanus. The smallest lethal dose of atropin for an adult is 130 milligrams, and of strychnine from 30 to 100 milligrams. "From this one can judge of the fearful weapons possessed by the bacteria in their poisons."

FERMI and PERNOSI draw the following conclusions from their studies of the tetanus poison:

(1) Agar-agar cultures are the most poisonons, next come those on gelatin, and lastly those in bouillon.

(2) Chickens, snakes, turtles, and tritons are immune to the poison.

(3) In the above-mentioned animals the tetanus poison may remain and retain its virulence for three days, and even longer.

(4) Filtrates from agar-agar and gelatin cultures are more resistant to heat than those from bouillon. Like the enzymes, the purer the tetanus poison the less stability does it possess.

(5) Dissolved in water, the tetanus poison is rendered inert by a temperature of 55° , but in the dry state it can be heated to 120° without loss of virulence.

(6) When the dried poison is mixed with ether or chloroform and heated to 80° , it is destroyed; but with amyl alcohol or benzol, a temperature of 100° is required to accomplish this result.

(7) Dissolved in water, this poison is destroyed by direct sunlight after an exposure of eight to ten hours (with the

highest temperature on a blackened thermometer at 56°), and after fifteen hours when the temperature did not exceed 37° .

(8) In the dry state the tetanus poison can be exposed to the direct sunlight for 100 hours without loss of virulence.

(9) Under the action of an electric current of 0.5 ampère, continued for two hours, the substance becomes inert.

(10) The poison is destroyed by the following substances: potassium permanganate, 50 per cent., for forty-eight hours; phosphotungstic acid, saturated solution, for twenty-four hours; lime water, saturated solution, for twenty-four hours; aseptol, concentrated, for twenty-four hours; kresol, concentrated, for twenty-four hours; lysol, concentrated, for twenty-four hours; hydrochloric acid, 25 per cent., twenty-four hours; butyric acid, 25 per cent., twenty-four hours; phosphoric acid, 25 per cent., twenty-four hours; oxalic acid, 4 per cent., twenty-four hours; tartaric acid, 1 per cent., twenty-four hours.

It is not destroyed by the following: antimony tartrate, 5 per cent., twenty-four hours; lead acetate, saturated, four days; magnesium oxid, forty-eight hours; chloroform, four days; acetic acid, twenty-four hours.

According to KITASATO, the tetanus poison is destroyed after twenty-four hours exposure to the following: tannin, 1.5 per cent. solution; paraphenolsulphuric acid, 2.5 per cent.; caustic lime, 0.08 per cent.; ammonia, 6.9 per cent.; caustic soda, 3.2 per cent.; barium hydrate, 1.0 per cent.; platinum chlorid, 0.4 per cent. After an exposure of one hour: gold chlorid, 0.5 per cent.; alcohol, 60 per cent.; methyl alcohol, 50 per cent.; amyl alcohol, 77 per cent.; carbolic acid, 1.5 per cent.; soda lye, 0.4 per cent.; iodine trichlorid, 0.5 per cent.; kresol, 1 per cent.

(11) Sulphuric oxide, oxygen, carbonic acid, carbon monoxid, methane, and hydrogen, even after from ten to fifteen hours, do not appreciably impair the poison.

(12) Gastric juice destroys the poison through the activity of the hydrochloric acid and not by virtue of the pepsin.

(13) Ptyalin, diastase, and emulsin have no action. The effect of trypsin has not been satisfactorily determined.

(14) Putrefactive germs do not destroy the poison.

(15) The living, but not the dead, intestines of guinea-pigs and cats destroy the poison.

(16) The living intestine of the chick does not destroy and does not absorb the poison.

(17) The poison may be eliminated by the kidneys and retain its properties in the urine.

(18) The poison is not a ferment.

The tetanus poison is a secretion of the bacterial cell and not a split product from constituents of the medium in which the germ grows. Its formation is due to synthetic process instituted in the bacterial cell, and it is found when the bacillus is grown in fluids that contain only asparagin and inorganic salts.

BRUSCHETTINI has studied the distribution of the tetanus poison through the body and its elimination in the following manner :

Animals were poisoned by injections of the substance prepared by TIZZONI and CATTANI, and just before death they were killed and bits of various organs rubbed up with sterilized water were injected into other animals. Emulsions from the liver and suprarenal capsules were invariably without effect, while those from the kidney were constantly poisonous. This is supposed to prove that the poison is eliminated by the kidney. The blood taken from the vena cava was found to be poisonous in three out of four experiments. When the injections were made under the skin the lumbar cord was active in four out of eight cases, and in all when the injections were made directly into the sciatic nerve. On the other hand, when the inoculations were made under the dura mater, the brain was found to be active while the lumbar cord remained inactive. From these experiments it is concluded that the poison not only circulates in the blood, but is deposited in the central nervous system. This author has more recently shown that the poison is eliminated by the kidneys. This he has demonstrated with the urine of men suffering from tetanus.

However, BRUNER in several cases of tetanus in man,

STERN in two, and BRIEGER in one case, were not able to induce tetanus in animals by injecting even large amounts of the urine of the patients. This does not cast doubt upon the accuracy of the report of BRUSCHETTINI; it only shows that the poison is not in all cases eliminated by the kidney in sufficient quantity to render the urine highly toxic. In a fatal case of acute tetanus VULPIUS failed to induce tetanus with the urine voided during life, but succeeded with that found in the bladder after death.

BUSCHKE accidentally pricked his finger-nail with a needle with which he had injected a virulent culture of the tetanus bacillus into a mouse. The wound was enlarged and disinfected. Half an hour later 2 c.c. of a 1 per mille solution of mercuric chlorid was injected into the tissue about the wound, and five days later 5 c.c. of Behring's antitetanic serum were injected into the thigh. Eight days after the serum treatment, headache, rheumatoid pains, and marked prostration appeared. BUSCHKE thinks that he immunized himself, but he admits that it is quite impossible to decide whether the symptoms were due to the infection or to the treatment.

BUSCHKE and OERGEL induced tetanus in guinea-pigs with blood serum obtained from a fatal case by venesection. With extracts from the liver, spleen, and spinal cord after death like results were also obtained in mice.

According to the studies of QUADT, the tetanic poison when injected directly into the blood circulates unchanged and unabsorbed for some hours. He also states that a much larger dose is required to induce tetanic symptoms when given intravenously than when given subcutaneously. Moreover, if a sublethal dose be given subcutaneously, the local tetanic symptoms are not intensified by the simultaneous intravenous injection of a second sublethal dose.

A. BABES prepared, from cultures made by V. BABES and PISCARIU in agar containing no pepton, an albumose which caused tetanus in animals.

FABER finds in a mixed culture a poisonous proteid body which resembles closely, so far as it has been studied, that of

TIZZONI and CATTANI. FABER lays much stress upon the arguments in favor of this substance being a soluble ferment. With this proteid, convulsive movements first appear and become very distinct in the muscles about the point of injection. In case very small amounts are employed the convulsive movements do not become general and the animal finally recovers.

PEYRAUD claims to have secured immunity in animals against "earth tetanus" by giving to them strychnia in gradually increased doses. NOCARD could not confirm this claim.

According to LEDANTEC, the poisonous arrows of the natives of the New Hebrides are prepared as follows: The points, which are usually made from human bones, are first covered with a vegetable resin, then smeared with the slime of swampy places.

LIERMANN found that material taken from the arm of a man who had died from tetanus, and who had been buried for two and one-half years, induced tetanus in animals. This would seem to show that the poison retains its virulence for a long time. In this material there were found nine kinds of bacteria, but none of these in pure culture, or in mixed culture, induced the disease. This he explained by the supposition that non-pathogenic bacteria may receive toxicogenic properties from the media (the dead body in this case) in which they grow.

With the tetanus bacillus widely distributed in the soil, it would not be surprising to find that material taken from the arm of a man, dead of any disease, after burial for two and one-half years, should induce tetanus in animals. Moreover, the failure to find the tetanus bacillus does not call for the supposition given by LIERMANN. The tetanus poison, without any bacilli, would produce the same effect.

RONCALI has tested forty different germs, some of them pathogenic and others non-pathogenic, in endeavoring to find one which would neutralize, either by its growth in the body or by the action of its products, the tetanus poison. His results were wholly negative. The tetanus poison was found to act more energetically in animals inoculated with other bac-

teria or treated with their products, and in no case was there any evidence of antagonism in action.

TUBERCULOSIS.—In 1865 VILLEMIN demonstrated that tuberculosis belongs to the infectious diseases. He induced the disease in the lower animals by feeding them upon tuberculous sputum and tissues. Before this time, tuberculosis had been generally regarded as an inherited disease, and it was supposed that all of those who “carried the taint in their blood” must succumb to the inevitable or escape by some lucky accident wholly beyond human control. When we remember that one-seventh of all deaths are due to this disease, we can appreciate the importance of learning everything that possibly can be known concerning its origin and spread. In 1868, CHAUVEAU, and a few years later COHNHEIM, experimentally confirmed the discovery of VILLEMIN, which must be regarded as one of the most important contributions to medical knowledge made during the nineteenth century. In 1878, TAFFEINER showed that the disease might be transmitted by the inhalation of infected dust. However, the nature of the infectious agent in tuberculous sputum and tissue remained unknown until 1882, when KOCH, after a most exhaustive research covering different manifestations of tuberculosis in man and some of the lower animals, announced the discovery of the specific bacterium of this disease. This work was so thoroughly done that practically every statement made by KOCH in his first report stands unchallenged to-day. The thirteen years that have elapsed since that time have each brought its confirmations of the facts then recorded. It is not within the province of this book to discuss in any detail the bacteriology of tuberculosis, and it must suffice to say that the causal relation of the bacillus tuberculosis to the disease is not now questioned by any competent authority. Every case of tuberculosis is due to infection from a preexisting case in man or beast. We are to concern ourselves wholly with the chemic poisons produced by this bacterium and by virtue of which the symptoms of the disease and death are

induced. We may be permitted, however, to call attention to the fact that in its later stages tuberculosis becomes a mixed infection and the chemie products of more than one bacterium constitute the causal factors in this form of poisoning.

KOCH's tuberculin, with which he hoped to cure the disease, is the crude poison formed by his bacillus and is known as tuberculin. The methods of preparing this poison are somewhat varied and we will mention some of them. KOCH prepared tuberculin in the following manner: Meat infusion containing 1 per cent. of pepton and from 4 to 6 per cent. of glycerin is placed in sterilized flasks with broad bottoms. The flasks are only partially filled in order that the surface of the fluid should be as great as possible. A small mass of a growth of tubercle bacilli is taken from a culture on glycerin-agar or blood serum and floated on the surface of the meat infusion in the flask. The flask is then placed in an incubator at 37°. The bacilli grow abundantly on the surface of the meat infusion, soon forming a thick yellowish-white layer on the entire surface. After about six weeks growth stops, the bacterial layer begins to break into pieces and these fall to the bottom of the flask. The culture is now evaporated to one-tenth of its volume on the water-bath. The concentration increases the per cent. of glycerin to from 40 to 50, and this ingredient prevents the growth of extraneous bacteria and renders the fluid permanent for an indefinite time. After filtration through porcelain, this fluid constitutes the crude tuberculin of KOCH. It will be seen that it must contain, in addition to the water and glycerin, any other unchanged constituent of the original meat infusion, any split products, if there be such, arising from the cleavage action of the bacilli on the components of the culture medium, and all the soluble constituents of the bacterial cells. Evidently the toxin in this impure form is not destroyed by the temperature of the water bath. Several ultimate analyses of this crude tuberculin have been made, but it must be evident from the statements just made concerning the complexity of its composition that such determinations are absolutely without value. It does

not contain any ptomaines or any other cyanogen bodies. A voluminous precipitate is produced on the addition of strong alcohol, and this precipitate contains the toxin. Since the toxin has not been isolated, its physical properties and chemie reactions remain for the most part unknown. The toxic substance is soluble and dialyzable.

BUJWID obtained tuberculin by extracting the growths of the tubercle bacillus on glycerin-agar tubes, heating to 100° for ten minutes, filtering through porcelain and concentrating at a low temperature. As thus prepared the fluid resembles very much the preparation already described.

Tuberculin may also be obtained from bacilli grown on potatoes. The freshly cut surfaces of the sterilized potatoes are washed with a 1 per cent. sterilized solution of sodium bicarbonate, then moistened with sterilized water containing 5 or 6 per cent. of glycerin. On potatoes thus prepared the bacillus grows quite abundantly at a temperature of 37°. After further development has ceased, the growths are extracted with water or water and glycerin.

Many attempts have been made to purify the toxin of these crude products, but up to the present time no satisfactory degree of success has been attained. KOCN obtained a white precipitate containing the toxin by the addition of 60 per cent. of alcohol to the crude tuberculin, but this precipitate is known to be a mixture of several bodies. HUNTER thought that he had separated the curative from the pyrogenic constituent, but the former, as he has obtained it, possesses no curative properties, and the latter is still a crude toxin. The "tuberculocidin" of KLEBS is obtained from crude tuberculin by fractional precipitation with alcohol.

The physiologic action of tuberculin is so pronounced and, at first, was believed to be so markedly *sui generis* that its worthy discoverer, and through him the greater part of the medical world, was for a short time led into the belief that a specific and sure cure for the greatest plague of man had been found at last. The grounds for this belief were founded principally upon the following effects observed in the action

of tuberculin: (1) Small doses, 1 milligram or even less, injected subcutaneously in an individual suffering from tuberculosis, caused a marked elevation of temperature. Similar doses injected in the same way into non-tubercular persons produced no appreciable effect. No effect followed the treatment with tuberculin of persons sick with other diseases than tuberculosis. Here then is a substance that has a specific action, a chemie body by the effects of which you can distinguish a tubercular from a non-tubercular individual. If all the cows of a large herd be treated with tuberculin and a record of the temperature be made for twenty-four hours before and for the same length of time after the treatment, it will be found that in some a febrile reaction—an elevation of one degree or more in temperature—occurs, while the temperature of the others remains unaffected. Now, if all be killed and examined, it will be found that those that have manifested the febrile reaction are tuberculous; while those that failed to react are not tuberculous. A similar test to this was made by KOCH on tuberculous and non-tuberculous guinea-pigs. No such effects had ever before been obtained by the employment of any therapeutic agent. It is small wonder then that KOCH and his colaborers were surprised at the results observed, and readily accepted and too speedily announced the belief that a specific cure for tuberculosis had been found. The grounds for this belief were strengthened by their observation of an additional evidence of the selective action of tuberculin. (2) Not only does tuberculin select tuberculous individuals by its action, but in the individual it selects for the demonstration of its most conspicuous effects the exact site of the tubercular lesion. If a man who has a lupus on the face receives a tuberculin injection in the back or in any other portion of his anatomy, the tissue about the lupus soon begins to show evidence of stimulation. It becomes hyperæmic, the margins of the sore begin to granulate, and if the treatment be continued the lupus often temporarily heals.

BAUMGARTEN draws the following conclusions from his

experiments with tuberculin on rabbits with inoculation tuberculosis :

It causes an exudative inflammation in the vascular tissue about the tubercle, and in this way the tuberculous tissue may be isolated and, when situated superficially, removed. In some cases, however, after the prolonged employment of the agent, the tuberculous tissue itself may, under the influence of the exudative fluid and the polynuclear leucocytes, break down and form abscesses. The bacilli themselves are in no way harmed by the use of tuberculin, and, after its constant employment for months, they retain their original form and lose none of their virulence. Some preparations seem to show that the bacilli multiply more rapidly when the injections are made, but a positive statement on this point is reserved until further studies have been made. It is certain, however, that the non-tubercular tissue of animals acquires no immunity against the disease from the injections. This is shown by the appearance of metastatic foci in animals in which from seven to twelve grams of the original lymph (an amount which would be equivalent to from seventy to one hundred and eighty grams in man) have been injected. It is further shown by the fact that in some animals treated subcutaneously tubercles have appeared at the point of injection.

PRUDDEN and HODENPYL summarize the results which they have obtained by the inoculation of animals with dead tubercle bacilli as follows: "These dead tubercle bacilli are markedly chemotactic. When introduced in considerable amount into the subcutaneous tissue or into the pleural or abdominal cavities they are distinctly pyogenic, causing aseptic, localized suppuration. Under these conditions they are capable, moreover, of stimulating the tissues about the suppurative foci to the development of a new tissue, closely resembling the diffuse tubercle tissue induced by the living germ. We have found that dead tubercle bacilli introduced in small numbers into the bloodvessels of the rabbit largely disappear within a few hours or days, but that scattering individuals and clusters may remain here and there in the lungs and liver, clinging to

the vessel walls for many days without inducing any marked changes in the latter. After a time, however—earliest in the lung, later, as a rule, in the liver—a cell-proliferation occurs in the vicinity of these dead germs, which leads to the formation of new multiple nodular structures bearing a striking morphological resemblance to miliary tubercles. There is in them, however, no tendency to cheesy degeneration and no evidence of proliferation of the bacilli, but rather a steady diminution in their number. It seems to us that the new structures originate in a proliferation of the vascular endothelium under the stimulus of the dead and disintegrating germs.” This work has been confirmed by others.

MAFFUCCI finds that cultures of the tubercle bacillus (from a mammal), when grown from one to six months on glycerin, blood serum, or liquid blood serum, and then sterilized by being repeatedly heated to from 65° to 70°, produces in guinea-pigs, when employed subcutaneously, a progressive marasmus, which terminates fatally within from fourteen days to five or six months. He also finds that eggs inoculated with sterilized cultures of the chicken tuberculosis bacillus produce chickens which are feeble and soon die of emaciation. In neither the guinea-pigs nor chickens could he find any tubercles. This author, unfortunately, does not state positively whether the bacilli employed in his experiments on guinea-pigs were obtained from man or some other mammal.

CROOKSHANK and HERROUN report the isolation of a ptomain and an albumose not only from artificial cultures of the bacillus, but also from bovine tuberculous tissue. The ptomain is reported as causing an elevation of temperature in tuberculous, and a depression in healthy, animals. “The albumose, whether obtained from pure cultivations of the bacillus or from tuberculous tissue, produced a marked rise of temperature in tuberculous guinea-pigs. On the other hand, in an experiment tried on a healthy guinea-pig, there was an equally well-marked fall of temperature.”

As early as 1888, HAMMERSCHLAG found a toxin among the products of the growth of this germ. More recently he

finds that as much as 27 per cent. of the cellular substance of the bacillus tuberculosis is soluble in alcohol and ether. In this extract there is, in addition to fat and lecithin, a poison which induces in rabbits and guinea-pigs convulsions followed by death. The part insoluble in alcohol and ether consists of cellulose and proteids. HAMMERSCHLAG has also prepared from cultures of this bacillus a "toxalbumin" which, when injected subcutaneously in rabbits, causes an elevation of temperature of from 1° to 2° , which continues for a day or longer.

ZUELZER has reported the isolation of a poisonous ptomain from agar cultures of the bacillus tuberculosis. He says that the injection of 1 centigram or less of this substance subcutaneously in rabbits or guinea-pigs causes, after from three to five minutes, increased frequency of respiration (to 180 per minute?) and an elevation of temperature of from 0.5° to 1° . He also reports marked protrusio bulbi as a constant symptom; the eyes become very bright and the pupils are dilated. From two to three centigrams suffice to kill rabbits, death occurring in from two to four days. The place of injection is reddened, and hemorrhagic spots are formed in the mucous membrane of the stomach and small intestine. In two instances from 15 to 20 cubic centimetres of clear fluid were found in the peritoneal cavity.

KITASATO treated fifty guinea-pigs that had been rendered tuberculous by subcutaneous inoculations with pure cultures of the bacillus with tuberculin. The beginning dose was 0.001 gram, and this was gradually increased to a maximum of from 0.15 to 0.2 gram. Five of the treated animals survived, and the average length of life of the treated animals exceeded by a few weeks that of the controls. It is a noteworthy fact that quite a number of the treated pigs developed and died of pneumonia. KITASATO attributes this to the existence of an epidemic of pneumonia among the animals of the laboratory at the time. However, if the treatment was not concerned in the production of the pneumonia, he should explain why none of the untreated pigs of the series developed pneumonia.

BUJWID also claims to have prolonged the lives of tuberculous guinea-pigs and rabbits by treating them with tuberculin. On the other hand, BAUMGARTEN and his students, also, CZAPLEWSKI and ROLOFF, failed to obtain any beneficial results by the tuberculin treatment of inoculated animals.

HERICOURT and RICHER claim to have vaccinated dogs against tuberculosis by the intravenous injection of the bacillus of avian tuberculosis. However, as this animal is not highly susceptible to this disease naturally, this statement should not be too readily accepted. TRUDEAU has succeeded in securing partial immunity in the rabbit by previous subcutaneous inoculations with the bacilli of bird tuberculosis. About one-fourth of the rabbits died from marasmus consequent upon the protective treatment. No tubercular lesions could be found in these after death. Those that recovered from the effects of the cultures of the avian bacilli were inoculated, along with controls, in the anterior chamber of the eye with the Koch bacillus.

The great majority of the reports on the curative properties of tuberculin has been unfavorable to the employment of this agent.

Recently (1895) two claimants of the discovery of a curative serum for tuberculosis have appeared. Both of these claimants are somewhat indefinite in their statements concerning the preparation of these serums, but are not equally reserved in their statements concerning the cures wrought. PAQUIN immunizes horses by methods not given, and "a horse treated properly three months may yield serum with immunizing power that will probably prove sufficient to arrest consumption in the first stage in three or four months, and sometimes less; and in the second stage in four to six months or a year."

MARAGLIANO does not think it desirable to give his method further than to state that he immunizes dogs, donkeys, and horses with a specially powerful tuberculin. He proves that the dogs are immune by subsequent intravenous inoculations of the bacillus. He is not sure that the donkeys and horses

are immune, but he considers this, after all, of no importance, because an animal may furnish an antitoxin, and remain itself susceptible. With these serums he treats and cures tuberculosis in man.

DIPHTHERIA.—That the LOEFFLER bacillus is the cause of the disease now known as “true diphtheria” no one can deny. The fact that this germ, although found only at the seat of inoculation, causes marked systemic disturbances, indicates that its action must be due to its soluble products. This was early recognized by LOEFFLER, who in 1887 attempted to ascertain the nature of the poison. A flask of bouillon containing pepton and grape-sugar was, three days after it had been inoculated with the bacillus, evaporated to 10 c.c., and this was injected into an animal, but was without effect. A second flask of the same material was extracted with ether, but this extract was also found to be inert. Next, some neutral beef broth was extracted with glycerin some four or five days after it had been inoculated with the bacillus. The glycerin extract, when treated with five times its volume of absolute alcohol, deposited a voluminous, flocculent precipitate, which was collected, washed with alcohol, dried, and dissolved in a little water. A further precipitation with alcohol and a current of carbonic acid gas secured a white substance, and the injection of from 0.1 to 0.2 gram of this, dissolved in water, subcutaneously in guinea-pigs, caused marked pain, followed by a fibrous swelling with hemorrhage into the muscles and oedema, terminating in necrosis. From these studies LOEFFLER concluded that the poison belongs to the enzymes.

ROUX and YERSIN found that bouillon cultures from which the bacillus had been removed by filtration through a Chamberland filter are poisonous, especially cultures that are four or five weeks old. The results obtained varied with the amount of the fluid, the species of animal, and the method of administration. The effects observed were a serous exudation into the pleural cavity, a marked, acute inflammation of the kidney, fatty degeneration of the liver, especially after injec-

tion into a bloodvessel, and œdematous swelling in the surrounding tissue after subcutaneous inoculation. In some instances, in dogs, rabbits, and guinea-pigs, paralysis, generally in the posterior extremities, followed. The action of the poison was found to be very slow, and, as a rule, death occurred days, and in some instances weeks after the inoculation, and was preceded by marked emaciation.

The cultures first employed were seven days old; older cultures (six weeks) contain more of the poison, and the symptoms appear within a few hours. In cultures especially rich in the poison, a small amount (from 0.2 to 2 c.c.) injected under the skin in guinea-pigs suffices to induce the symptoms. Mice and rats are markedly insusceptible, but succumb to large doses.

Heating to 100° for twenty minutes renders the poison inert, and a temperature of 58° maintained for two hours markedly lessens its virulence.

The poisonous substance is precipitated by absolute alcohol, and is carried down mechanically on the addition of calcium chlorid to the filtered cultures. These investigators agree with LÖEFFLER that the poison belongs to the enzymes. The great toxicity of this substance is indicated by the statement of ROUX and YERSIN that 0.4 milligram suffices to kill eight guinea-pigs or two rabbits, and that 2 centigrams of the calcium chlorid precipitate, containing about 0.2 milligram of the pure poison, will kill a guinea-pig within four days.

BRIEGER and FRAENKEL made a study of the chemie products of the Loeffler bacillus. They employed cultures of bouillon and pepton containing from five to six per cent. of glycerin, and others containing ten per cent. of sterile, fluid blood serum. The latter were found to be most suitable. In these the bacilli grew most abundantly. In all cases they confirmed the statement of ROUX and YERSIN that the cultures, at first alkaline, became strongly acid, and finally again alkaline, with the exception that the glycerin cultures remained acid.

For the removal of the bacteria two methods were employed.

First the bacilli were destroyed by heat. When a temperature of 100° was employed the cultures were rendered inert, but it was found that exposure for from three to four hours to a temperature of 50° was sufficient to destroy the germs, while the virulence of the chemie products was not affected. The second method of removing the bacteria consisted of filtration through a Chamberland filter. The germ-free filtrate could be heated to 50° without loss of toxicity, while a temperature of 60° rendered it inert. In the majority of the experiments the filtration method was used, and in this way a large quantity of a poisonous fluid of uniform strength was obtained.

Varying amounts of this fluid were used upon animals, mostly guinea-pigs and rabbits, and it was found that the effects varied with the quantities employed and the methods of administration. The symptoms appeared most promptly when the injections were made directly into a bloodvessel. Of four rabbits which were given subcutaneously respectively 1, $2\frac{1}{2}$, 5, and 10 c.c. of the filtrate on December 28th, the first died January 4th; the second, January 2d; the third, December 31st; and the fourth, December 30th. In all cases in which death did not occur too early, paralysis appeared. The limbs were first paralyzed, and this was true whether the fluid was administered intravenously or subcutaneously. The post-mortem appearances were identical with those observed after inoculation with the bacillus, with the exception of the absence of the pseudo-membrane. After subcutaneous injection there was a gelatinous, grayish-white, sometimes reddish, cedematous fluid formed at the point of injection; and, after larger doses, necrosis. In cases in which death was delayed, there were effusions in the pleura, fatty degeneration of the liver, and inflammation of the kidneys. Especially marked were these cellular changes in rabbits which were treated with small amounts intravenously.

BRIEGER and FRAENKEL conclude this part of their report with the following statement: "We have shown that the Loeffler diphtheria bacillus produces in its cultures a poisonous, soluble substance, separable from the bacteria, which

causes in susceptible animals the same phenomena that are induced by inoculation with the living microorganism. We have further shown that this substance is destroyed by a temperature over 60° , but that it can be heated to 50° , even in the presence of an excess of hydrochloric acid, without being destroyed. This last fact is contrary to the assumption that the chemie poison of the diphtheria bacillus is a ferment or enzyme."

The fluid was tested for basic products, but with wholly negative results, except that small amounts of creatinin and cholin were found. It was also distilled at from 20° to 35° in a vacuum, and the distillate was found to be inert.

The poisonous substance was found to be insoluble in alcohol, soluble in water, and non-dialyzable. It was precipitated by saturation with ammonium sulphate.

The substance was obtained by allowing the geru-free filtrate, after being rendered feebly acid with acetic acid, to fall into a large volume of absolute alcohol. It was purified by repeated solution in water and precipitation with alcohol. It contains a large amount of sulphur, and responds to the biuret and Millon tests. It is, therefore, classified among the albumins. Since it is not precipitated by saturation with magnesium sulphate at 30° , it cannot belong to the globulins. The fact that it is precipitated by saturation with ammonium sulphate, and that it does not dialyze, shows that it is not pepton. It is, therefore, classified by BRIEGER and FRAENKEL among the albumins, and is designated as a "toxalbumin."

This proteid induces in animals all the symptoms and post-mortem appearances which have been mentioned as following the administration of the filtered cultures. It is to be noted that the injection of small quantities of this proteid ($2\frac{1}{2}$ milligrams per 1 kilogram of the body-weight of the animal) does not produce its effects until after the lapse of weeks, and possibly months. This peculiarity in action distinguishes this class of substances from all other chemie poisons, and it has received as yet no satisfactory explanation. There is no reason for believing that the body obtained by BRIEGER and FRAENKEL

is chemically pure, and until it has been obtained in this condition we can only speculate concerning its true nature.

It should be remarked that the Loeffler bacillus shows not only marked morphologic variations, but that it is very variable in its virulence, some cultures having been obtained that are wholly without effect upon animals. From cultures of this kind BRIEGER and FRAENKEL prepared a non-poisonous albumin differing in its ultimate composition and in many of its chemie reactions from the poisonous one.

FRAENKEL has been unable to secure immunity in animals against diphtheria by the employment of small doses of the "toxalbumin." If the dose is large enough, the animal dies. If it is smaller, the animal seems to become more susceptible and succumbs more readily to inoculations with the germ. While this is true of the filtered culture, it is not the case with that which has been sterilized by heat. FRAENKEL finds that if from 10 to 20 c.c. of a culture of the bacillus three weeks old, which has been heated for one hour at from 65° to 70°, be injected under the skin of the abdomen of guinea-pigs, immunity against subsequent inoculation with the virulent germ is secured, provided that the inoculation is not made earlier than the fourteenth day after the treatment with the sterilized culture. He thinks that the culture contains two specific albumins, one of which is poisonous, while the other gives immunity. The former is destroyed by a temperature of from 65° to 70°, while the other retains its characteristic properties. He admits the possibility that the poisonous albumin may be converted into the other form by the high temperature. He finds that the modified culture, which gives immunity, is of no service for therapeutic purposes, and that if an animal be treated with it directly after inoculation with the germ, death is not retarded, but is hastened. From these experiments he concludes that the vaccination albumin at first lessens, and subsequently increases the resistance of the animal. We now know that immunity may be and is secured with the diphtheria toxin. The diphtheria curative serum is prepared in this way, as will be seen later. There is no reason for believing in

FRAENKEL's theory that the bacillus forms a special immunizing substance.

SPRONCK and his students have confirmed the above statements concerning the toxicity of the germ-free cultures of this bacillus. They have also called attention to the albuminuria following the employment of this poison. In the urine they find casts, white, and sometimes red, blood-corpuscles. Microscopic examination of the kidney after death shows the same changes that are observed in the diphtheritic nephritis of children. BABES also finds that the germ-free cultures produce the parenchymatous degenerations of the internal organs which are found in the human body.

TANGL has shown that the chemic poison is formed in the body as well as in culture-flasks. A large piece of pseudo-membrane was macerated in water in an ice-chest for twenty-four hours, and then filtered through porcelain. The filtrate, injected into animals, produced all the symptoms that had been obtained by a similar employment of artificial cultures. TANGL also observed that in some cases in which the animals were inoculated with the sterilized culture through the mucous membrane a pseudo-membrane formed at the point of injection. The diphtheria poison has also been found in the tissues, blood, and urine.

DZIERZGOWSKI and REKOWSKI have attempted to ascertain the nature of the chemic products of the diphtheria bacillus in the following manner: In the first experiment four flasks, each containing 2 litres of a solution of commercial pepton in water, were employed. After sterilization two flasks were inoculated with the bacillus, while the other two were kept as controls. All stood for six weeks at a temperature of 36.5° . Then the contents of one diphtheria and one control flask were distilled separately to half the volume, and the distillates redistilled and evaporated. When these residues were freed from ammonium chlorid with absolute alcohol, that from the inoculated flask left a substance soluble in water and fatal to a rabbit in fifteen minutes, while that from the control flask had no poisonous properties. It was also found that the

bacillus did not produce volatile acids, aromatic oxyacids, indol, skatol, leucin, tyrosin, or cadaverin.

The albumoses precipitated by absolute alcohol in the companion flasks gave substantially the same chemie reactions, but that from the inoculated, in doses of two-tenths of a gram given subcutaneously, killed guinea-pigs, while that from the other flasks in double this amount had no effect. The substances soluble in alcohol from both flasks were not poisonous.

Attempts were made to obtain the volatile body in large amount and chemically pure, but without success. In the purest form obtained 0.15 gram sufficed to kill a guinea-pig within eight minutes.

Further experiments demonstrated that the diphtheria bacillus slowly decomposes sugar, forming small amounts of formic and paralactic acids. Amylodextrin and fat were not altered, and glycerin only slightly. The authors attempt to explain theoretically how the comparatively inert albumoses of the culture-medium are changed by the bacillus into poisonous albumoses. However, there is no proof that any such change is wrought, or that the poisonous substance in the alcoholic precipitate is an albumose at all. The alcoholic precipitate from the inoculated flasks contained, in addition to the albumoses, the cellular toxin of the bacillus, and to this the effects were due.

SUPPURATION.—As early as 1879, LEBER concluded from his observation on infective keratitis that the aspergillus must produce certain soluble products which diffuse through the cornea and set up an inflammatory action in the adjacent vascular tissue. In 1882 he showed that suppuration could be induced by the introduction of sterilized mercury and copper, and that the pus formed is free from germs. In 1884 he induced suppuration by the injection of cultures of the staphylococcus pyogenes aureus which had been sterilized by being boiled for hours. In 1888 the same investigator reported that he had found an alcoholic extract of the dried staphylococcus to be highly pyogenetic. From this extract he has prepared

a crystalline body which he calls phlogosin. This substance is readily soluble in alcohol and ether, sparingly soluble in water, and it crystallizes in needles. The crystals can be sublimed, leaving no residue, and the sublimate, which forms in rosettes, still possesses the pyogenetic properties. Alkalies precipitate this substance from its solution in amorphous granules, which dissolve in acids, forming crystalline salts. LEBER refers to the observation of the botanist PFEFFER, who found that vegetable cells are attracted by certain chemical substances, and adopts the term chemotactic action (*chemotactische Wirkung*) to indicate the property of certain chemical agents of attracting leucocytes.

As has been stated, BUCHNER has found that the cells of many bacteria contain pyogenetic proteids. The amount of these substances in the cells varies with the kind of germ, and some species (the *bacillus prodigiosus*, for instance) seem to contain no such bodies. The *bacillus pyocyaneus* contains a large quantity of the proteid, and is suitable for lecture demonstration. The germs are taken from potato cultures and rubbed up with water. Then they are treated with about fifty volumes of a 0.5 per cent. solution of caustic potash. This forms in the cold a mucilaginous mass which dissolves at the temperature of the water-bath. After being heated for some hours the fluid is filtered through a number of small filters; the first portions should be refiltered. The filtrate is a greenish fluid (pyocyantin) which by the careful addition of acetic or hydrochloric acid (an excess is to be avoided) forms a voluminous precipitate (pyocyaneus proteid). This precipitate should be collected on a filter, washed with water, then suspended in water, and a few drops of a soda solution added, when a dark-brown fluid, with a tendency to gelatinize in the cold, containing about 10 per cent. of the proteid, is obtained.

13.254 grams of the moist bacteria yield 1.44 gram of dry bacterial substance, and this after the treatment given above furnishes 0.2739 gram of dry proteid = 19.3 per cent. This proteid leaves 11.52 per cent. of ash, which contains phosphoric acid, but consists principally of sodium chlorid.

Much smaller amounts of proteid were obtained from other germs, but the Eberth germ, bacillus subtilis, lactic acid bacillus, red bacillus from potato, and staphylococcus pyogenes aureus furnished considerable quantities.

The chemotactic properties of these proteids were tested in the following manner: The dissolved proteid was placed in a spindle-shaped glass tube, and the tubes, sterilized by prolonged boiling, were introduced under the skin on the backs of rabbits with antiseptic precautions, and the ends of the tubes broken off subcutaneously.

After from two to three days the tubes were removed and found to contain, in addition to some of the proteid, several millimetres of fibrinous pus, which was examined microscopically and by the preparations of cultures, which invariably remained sterile. The proteid of the Eberth bacillus was found to have specially marked pyrogenetic properties.

Similar experiments were made with the following crystalline substances: the butyrate and valerianate of ammonia (each 1 per cent. solution), trimethylamin (2 per cent.), ammonia (2 per cent.), leucin, tyrosin and glyceol (1 per cent.), urea (5 per cent.), and urate of ammonia and skatol (1 per cent.). Glyceol and leucin only were found to have the chemotactic action, and with these this action was but slight compared with that of the bacterial proteids.

The next experiments were made with the object of ascertaining whether or not proteids similar to those derived from the bacteria would cause a like effect. The bacterial cellular proteids resemble very closely vegetable casein, some of which was prepared from wheat gluten and tested as above. This proteid was found to be possessed of marked chemotactic properties. The subcutaneous injection of sterilized preparations of wheat-flour and ground peas were also found to cause suppuration. Negative results were obtained with starch and solutions of disodium hydric phosphate. From this it is concluded that the active agent in the flour is its casein.

Pepton was employed without effect, while gelatin was found to act energetically. Alkaline albuminates were prepared

from muscle, liver, lungs, and kidney by treating finely divided portions of these organs with potash and proceeding as in the preparation of the bacterial proteids. All of these caused the formation of pus, and the preparations from the liver were found to be specially potent.

Similar preparations from blood and egg-yolk were active, while those from fibrin and the white of egg had no effect. Hemi-albumose was also found to be active, and this fact is placed in contrast with the negative result obtained with pepton.

One of the most interesting results was obtained by the daily injection of a chemotactic proteid directly into the blood. Before the first injection the proportion of white to red corpuscles was 1 : 318; on the second day, 1 : 126; on the third, 1 : 102; on the morning of the fourth, 1 : 73; on the afternoon of the fourth, 1 : 38. After this there was no further increase. The absolute number of red corpuscles remained unchanged, while the absolute number of the white multiplied sevenfold. The white corpuscles were on the first days often found in groups of from two to four, and later, of from ten to twenty. This seems to demonstrate that these substances cause an increased production of leucocytes. General leucocytosis was induced by the similar employment of vegetable casein and an alkaline albuminate prepared from the muscles of a calf.

Finally, BUCHNER tested the action of this proteid upon himself. One cubic centimetre of a very dilute solution, containing 3.5 milligrams of the solid proteid, was injected under the skin of the forearm with antiseptic precautions. Two hours later there was marked pain along the lymphatics, especially localized in the elbow and axilla. The temperature showed no marked elevation (only 37.8°). On the following day there were marked erysipelatous redness and swelling extending for some inches about the place of injection, and accompanied by severe pain. The inflamed area felt hot, and projected distinctly above the surrounding surface. The lymphatics of the arm appeared like red cords. On the third day the swelling and redness were more marked, and extended

from the wrist to the elbow. On the fourth day the symptoms began to recede. Here we have clinically a perfectly typical erysipelas with lymphangitis, and BUCHNER claims that all the cardinal symptoms of inflammation—*rubor*, *calor*, *dolor*—could not be produced without involvement of the solid tissues.

Similar, but less marked, symptoms were induced by the injection of a dilute solution of vegetable casein.

BUCHNER states that bacteria will not cause inflammation unless they be broken down. The pyogenetic substance contained within the bacterial cell can have no chemotactic action until the cell disintegrates. Thus, the anthrax bacillus contains a pyogenetic substance, but no pus is formed in mice with anthrax, because there is no destruction of the bacilli. This pyogenetic proteid of the anthrax bacillus, however, manifests its action in malignant pustule.

These experiments are of the greatest interest. We must say, however, that it is possible that the bacterial cellular proteid may be modified by the treatment to which it has been subjected in these experiments. We do not as yet know enough about the nature of this proteid to say that its nature and its action are not altered by being heated for hours with an alkali. However, accepting BUCHNER's work, it throws much light upon processes which have heretofore been but imperfectly understood.

Many non-pathogenic germs may grow in wounds and by elaborating their poisons may influence and increase the general intoxication. BRUNNER has found the *proteus vulgaris* growing in a wound, and it is well known that the products of this germ are powerful poisons.

NANNOTTI, after treating animals with sterilized pus, states the following conclusions:

1. Sterilized pus has substantially the same toxic properties as sterilized cultures of the staphylococcus.

2. Repeated injections of sterilized pus induce chronic intoxication and marasmus.

3. Injection under the skin causes a specially grave form of poisoning.

4. The symptoms and pathological lesions caused by these injections correspond with those observed in men suffering from chronic suppuration.

THE SUMMER DIARRHOEAS OF INFANCY.—In a paper published in 1888, VAUGHAN stated that the microorganisms which produce the catarrhal or mucous diarrhoeas of infancy are probably only putrefactive or saprophytic in character, and that they prove harmful by splitting up complex molecules and forming chemic poisons, but those that cause the choleraic form or serous diarrhoeas are more than putrefactive, they are pathogenic. At that time it was generally believed that a specific germ would be found, but the truth of the above statement is being made more manifest with every experimental study of the subject. Able and diligent bacteriologists, among whom BOOKER, in this country, and ESCHERICH, in Germany, deserve special mention, have made a careful study of the bacteria found in the intestines and stools in these diseases, and all agree that no specific organism has been found. BOOKER has reported the isolation of more than thirty kinds. In true cholera infantum the proteus group of bacteria was found in fifteen out of nineteen cases, but in the ordinary diarrhoeas there is no constancy in the species present. Germs that are frequently found one year are rarely seen in the cases observed the next summer. This has been the experience of all who have studied the bacteria of the summer diarrhoeas of infancy. VAUGHAN has studied the chemical products of the germs x, a, and A of Booker's list in the following manner and with the results as stated below.

Of these germs, BOOKER makes the following statements:

"x was found almost as a pure culture in the feces of a fatal case of diarrhoea. a was strongly pathogenic when tested last winter. A was isolated last summer; liquefies gelatin, and belongs to the proteus group."

Beef-tea cultures of each of these germs were made and kept in an incubator at 37° for forty-eight hours. At the expiration of this time these cultures were used for inoculating

flasks of sterilized beef-broth. Eight flasks, each containing about ten ounces, were employed for each germ. These cultures were kept in the incubator at 37° for ten days. They were then twice filtered through heavy Swedish filter-paper. The second filtrate was allowed to fall into a large volume of absolute alcohol feebly acidified with acetic acid. A voluminous, flocculent precipitate resulted in each case. After the precipitates had subsided the supernatant fluid was decanted. The precipitates were then treated with distilled water, in which those from x and a were soluble, while that from A proved insoluble. A large volume of absolute alcohol was again added, and the mixture allowed to stand for four days. The precipitates from x and a completely subsided, leaving the supernatant fluids perfectly clear; but in the case of A the subsidence was not complete. The precipitates were collected, by decantation and filtration, on porous plates, and dried over sulphuric acid. These substances are proteid in composition, but differ from known proteids and from one another. That from x is slightly yellow, as seen deposited in the alcohol, but becomes grayish on exposure to the air. It is readily soluble in water, from which it is not precipitated by heat or nitric acid, singly or combined.

It gives the biuret and xantho-proteid reactions. It is precipitated by saturating its aqueous solution with ammonium sulphate, and therefore cannot be classed with the peptons. Sodium sulphate and carbonic acid fail to throw it down from its aqueous solution, consequently we must say that it is not a globulin.

This leaves us with no other choice than to place it among the albumins, but we must admit that it possesses properties which do not belong to the known albumins.

The proteid prepared from cultures of the germ a is, as seen under the alcohol, very light, flocculent, and perfectly white, but so soon as it is brought in contact with the air it begins to blacken, and finally dries down on the porous plate in black scales.

It possesses the same general properties in regard to the

action of solvents and other reagents which were found to be possessed by the proteid obtained from cultures of *x*.

The proteid of *A* is peculiar, inasmuch as it is practically insoluble in water.

These three proteids are highly poisonous. When injected under the skin of kittens or dogs they cause vomiting and purging, and, when employed in sufficient quantity, collapse and death. Post-mortem examination shows the small intestine pale throughout and constricted in places. The heart has been invariably, so far, found in diastole and filled with blood. The following brief notes from the record of experiments will illustrate the nature of the symptoms and the post-mortem appearances :

A small amount of proteid from bacillus *x*, dissolved in water, was injected under the skin on the back of a kitten about eight weeks old. Within one-half hour the animal began to vomit and purge, and death resulted within eighteen hours. The small intestines were pale, contracted in places, and contained a frothy mucus. The stomach was distended with gas and contained yellowish mucus. The liver was normal, the spleen and kidneys congested, and the heart distended.

Another kitten was treated with the proteid from bacillus *a*, dissolved in water. The vomited and fecal matters in this case were green. The animal died after fifteen hours, and presented appearances practically identical with those mentioned above.

A third kitten was treated with some of the proteid of bacillus *A*, suspended in water, and presented substantially the same symptoms and post-mortem appearances.

A fourth animal was treated in the same manner as the above with a proteid prepared from some canned meat. This was done as a control on the above experiments, and the kitten remained unaffected. This experiment demonstrates the fact that the poisonous properties are peculiar to the bacterial proteids.

Concerning the amount of one of these proteids necessary

to produce a fatal result in the animals experimented upon a few experiments have been made.

Under the skin on the back of a guinea-pig VAUGHAN injected 10 milligrams of the dry-scale proteid from bacillus a. This caused death within twelve hours. Of two kittens treated with 15 milligrams each of the a albumin, one died after forty-eight hours and the other recovered after two days of purging and vomiting. Two dogs, of about five pounds weight, had each 40 milligrams, and, after serious illness of two days' duration, speedily recovered.

During these two days of vomiting and purging the dogs were constantly shivering, as with cold, but the rectal temperature stood at from 102.5° to 103.5° F.

There was in no case any sign of inflammation at the point of injection.

Plate cultures have been made from the proteids themselves and from the blood, liver, spleen, and kidneys of some of the animals killed with the proteid, and these plates have remained sterile, thus demonstrating that no germ has been introduced into the animal along with the chemie poison.

What conclusions may we draw from these facts when considered in connection with the results of the labors of BOOKER and ESCHERICH? We will formulate our ideas in the following propositions:

1. There are many germs, any one of which, when introduced into the intestines of the infant, under certain favorable conditions, may produce diarrhœa.

As has been stated, many different germs have been found in the intestines of infants suffering from summer diarrhœa, and we now find that three species of these are capable of producing chemie poisons, which induce effects substantially identical with the symptoms observed in the infants, and it is not unreasonable to suppose that many other of these germs produce similar poisons.

2. Many of these germs are probably truly saprophytic.

A germ growing in the intestine does not necessarily feed upon living tissue. The food in the duodenum before absorp-

tion has no more vitality than the same material in the flask. Moreover, the excretions poured into the intestines from the body are not supposed to be possessed of vitality. A germ which will grow upon a certain medium in the flask and produce a poison will grow on the same medium in the intestine and produce the same poison, provided it is not destroyed by some secretion of the body.

3. The only digestive secretion which is known to have any decided germicidal effect is the gastric juice; therefore, if this secretion be impaired there is at least the possibility that the living germ will pass on to the intestine, will there multiply, and will, if it be capable of so doing, elaborate a chemie poison which may be absorbed.

There is no longer any doubt that the acid of the gastric juice has a marked germicidal effect upon many of the micro-organisms.

VAUGHAN has found that an exposure to a two-tenths per cent. solution of hydrochloric acid for half an hour will destroy Eberth's germ and two poison-producing bacilli which he has isolated from drinking-water which was believed to have caused typhoid fever. Although the germicidal effect of this acid has not been tried on the bacteria under consideration, doubtless it will be found to be considerable.

The chief reason why the breast-fed child has a better chance for life than the one fed upon cow's milk lies in the fact that the former gets its food germ-free; but a second reason is to be found in the larger amount of acid required to neutralize the cow's milk, as has been pointed out by ESCHERICH. The gastric juice is the physiological guard against infection by way of the intestines.¹

It is also possible that some of the secretions poured into the intestines have germicidal properties, or that the cells, in

¹ It has been said that this statement cannot be true, because there are other acids which are more powerful germicides than hydrochloric acid, but there is no force in this argument. The question is not whether the stomach is supplied with the very best germicide, but whether it is supplied with any at all. The human eye may not be a perfect mechanism, but it is man's only organ of vision.

absorbing the poisonous proteids, may to a limited extent so alter them that they are no longer poisonous, or that in a perfectly normal condition the liver may be able to prevent these poisons from entering the general circulation without change. These are all possibilities, which science at some time in the future will investigate.

4. Any germ which is capable of growing and producing an absorbable poison in the intestine is a pathogenic germ.

It is not necessary that a germ be capable of growing and causing disease and death when injected under the skin or into the blood in order to establish its right to rank with the pathogenic germs. In the blood the organism is acted upon by a wholly different fluid from that with which it is surrounded in the intestine, and the germicidal properties of the blood have been unquestionably demonstrated.

5. The proper classification of germs in regard to their relation to disease cannot be made from their morphology alone, but must depend largely upon the products of their growth.

As has been stated, three microorganisms, differing sufficiently to be recognized as of different species, produce poisons, all of which induce vomiting and purging, and, when used in sufficient quantity, death. Morphologically these bacilli may not be closely related, but physiologically they are near akin.

If these deductions be true, we will try to avoid the introduction into the alimentary canal not only of the so-called specific pathogenic germs, but of all toxicogenic microorganisms.

BAGINSKY and STADTHAGEN have obtained from cultures of the "white liquefying bacterium" of the former a poisonous proteid which produces in mice, after about five hours, slight dyspnoea. The coat becomes rough, the animal sits with drooping head, and when forced to move does so sluggishly, but without any evidence of paralysis. The marked apathy increases, and death results after two or three days. Section shows an infiltration about the place of injection, congestion

of the spleen, liver, and peritoneum. The intestine is hyperæmic throughout its entire length, and its upper portion contains a reddish-brown fluid.

From cultures of the same bacterium BAGINSKY and STADTHAGEN have also obtained a poisonous ptomain, which is probably identical with one found by BRIEGER in putrid horseflesh, and which has the formula $C_7H_{17}NO_2$.

That tyrotoxicon is one of the causes of the violent choleraic diarrhoea of children there can scarcely be a doubt. The symptoms induced by the poison cannot be distinguished from those of the disease. The post-mortem appearances are very much alike, if not identical, and the poison has been found in a sample of milk a part of which had been given to a child not more than two hours before the first symptoms of a violent attack of the disease made themselves manifest.

FLÜGGE has made a very important contribution to our knowledge of the relation of the bacteria of milk to the summer diarrhoeas of infancy. He has studied these bacteria with special reference to their toxicogenic properties. He finds four anaerobic bacilli frequently present in milk. Two of these (III. and IV.) produce poisons. A milk culture of bacillus III. was freed from germs by filtration through a Berkefeld filter and injected subcutaneously in mice in doses of from 0.3 to 0.6 c.c. The animals died after from three to fifteen hours, and section showed marked hyperæmia of the intestines and transudates in the peritoneal and pleural cavities. Five c.c., given intra-abdominally, kill guinea-pigs within from fifteen to twenty-four hours. In these, also, the intestines are engorged and the abdominal cavity is filled with a serous transudate.

Filtered cultures of bacillus IV. caused in guinea-pigs whining, staggering, trembling, and marked dyspnoea, from which recovery followed. Cultures thirty-six hours old and those fourteen days old showed no difference in effects.

These germs cannot be considered as harmless. Little danger is to be expected from b. IV., because milk in which it develops has a most disagreeable odor, and consequently is

not likely to be taken. B. III. is only occasionally found in milk. Summer diarrhœa can scarcely be attributed to these anaerobic germs, and yet they cannot be regarded as harmless. It is especially worthy of mention that anaerobic bacteria are present in every sample of milk, that some of them are not destroyed by boiling, and that they grow better at from 30° to 37° than they do under 22°.

Of the peptonizing bacteria, twelve species were isolated and studied. On the addition of a pure culture of one of these germs to sterile milk a transparent zone forms under the layer of cream, and extends deeper and deeper with more or less rapidity according to the species and abundance of the organisms until the whole of the casein has been digested. The milk has the bitter, irritating taste characteristic of peptons. However, during the first days of this growth the taste is not sufficiently developed to attract attention. These germs grow most rapidly when supplied with air; therefore their development goes on faster in open than in closed vessels. Milk containing millions of these peptonizing bacteria is apparently perfectly normal and will be readily taken by infants. Milk containing them may be kept at 100° for two hours or even for a longer time without destroying their spores. Cultures kept at from 24° to 44° grow with enormous rapidity.

Different investigators who have tested the nutritive value of various peptons and albumoses on the lower animals and on healthy and sick men are unanimous in the verdict that the long-continued employment of these preparations causes in both men and dogs severe intestinal irritation. ZÜNTZ noticed that dogs fed on peptons suffered from an abundant, watery diarrhœa, and eliminated from three to six times as much nitrogen unused as those fed upon meat. MUNK obtained like results. PREIFFER induced in himself and another man intestinal irritation and diarrhœa by large doses of Koch's pepton. NEUMEISTER states: "By long-continued use of these preparations, symptoms of marked irritation and injury to the intestines uniformly resulted, and consequently

the prescription of albumoses in disease can scarcely be regarded as rational." The reason why partially digested milk has been so well borne by children is due to the fact that but little pepton is formed. Certainly from all that we know of the action of pepton the long-continued employment of peptonized milk in the feeding of infants cannot be recommended, and the presence of peptonizing bacteria in the milk cannot be regarded as harmless.

Of the twelve peptonizing germs in milk studied by FLÜGGE, nine failed to show any toxic action. These do not produce toxins, and any harmful effects that they might induce must be due to the peptons. Dogs fed upon cultures of these germs failed to manifest any untoward symptoms. However, no positive conclusion can be drawn from this negative evidence, because the feeding was not continued for a sufficient length of time.

"Wholly different results were obtained with the peptonizing bacteria Nos. I., III., and VII."

"The two-days-old cultures of No. I. induce in frogs, on the injection of two c.c. into the dorsal lymph-sac, first slowness of motion and reflexes, after an hour paralysis of the extremities and complete loss of reaction, and after four hours death. Mice die after from five to six hours from the subcutaneous injection of one-half c.c. With the exception of lack of voluntary motion and tardiness of reaction, no symptoms are manifest. Guinea-pigs, that have received about five c.c. intra-abdominally, lie on the side and have marked dyspnoea; the abdomen is retracted, and handling them causes pain. Death results after from four to seven hours. Section shows hyperæmia of the kidneys, and the peritoneum and serous coat of the intestines are markedly reddened. Nothing else of interest is found. Dogs drink the milk cultures with relish and in large quantities. After one hour severe diarrhoea sets in with a movement every five minutes. When fed with normal milk recovery follows."

"Two-days-old milk cultures of No. III. induce in frogs and mice no symptoms. Guinea-pigs and rabbits after re-

ceiving intra-abdominal or intravenous injections remain quiet in their cages, respond feebly to irritation, but gradually recover. The cultures, when fed to puppies, induce sharp diarrhœa and apparently severe pain in the abdomen. One of the puppies showed on the second day progressive exhaustion, paralytic weakness of the extremities, and fall in the temperature. He died on the third day. Section showed hyperæmia of the kidneys, nothing else worthy of note."

"Bacillus No. VII. injected in milk cultures in frogs, mice, and guinea-pigs had no marked action. When the culture was filtered through a Chamberland filter and concentrated in vacuo to one-fifth its volume, it killed mice and guinea-pigs when injected in doses of six-tenths and five c.c. respectively. Death, which followed in from six to twelve hours, was preceded by dyspnœa and convulsive movements. Section showed nothing characteristic. Even the unconcentrated milk cultures acted powerfully when fed to puppies. After feeding for one or two days, profuse diarrhœa set in, but disappeared the next day (the feeding being discontinued?). The diarrhœa was accompanied by great emaciation, weakness of the extremities, and tottering gait. As soon as the use of the cultures was discontinued, and ordinary milk given, improvement began and continued to complete recovery. Two puppies, after recovery, were again fed with the cultures. After a short time the profuse diarrhœa with its accompanying symptoms reappeared."

"The effect of bacilli Nos. I., III., and VII. on the intestines are scarcely attributable to the peptons. Bacilli V. and X. produced peptons much more energetically, without inducing the symptoms. On the other hand, the highly virulent bacillus III. produced the least peptons. Furthermore, the symptoms that followed the injections were not those that are caused by the injection of peptons. We failed to find on section the ecchymoses and hemorrhagic spots that are commonly observed after pepton injections. At all events, each of these bacteria forms poisons that are not found in cultures of the other peptonizing bacteria. Whether special kinds of peptons

are formed or the poisons that are formed have nothing to do with peptons, cannot yet be determined. I have undertaken a closer study of these bacterial poisons, but this work is accompanied by no slight difficulties, which are greatly increased by the fact that milk must be the basis of the culture medium. My investigations in this direction, also the possible relation of these poisons to the (angeblich von bac. butyrius gebildet¹) tyrotoxicon of VAUGHAN, must be deferred to a later publication. For the present purpose of this article, the more exact knowledge of these poisons is not necessary. It has been shown that in ordinary market milk three peptonizing bacteria are frequently found, pure cultures of which in milk induce serious poisonous effects in animals, and especially when fed to puppies cause a profuse diarrhoea, sometimes leading to a fatal termination."

FLÜGGE's most valuable article continues with the detail of experiments on the sterilization of milk, and should be read by all who are interested in the subject.

TYPHOID FEVER.—In 1880, EBERTH discovered a bacillus which he believed to be the cause of typhoid fever, and this belief has been quite generally accepted. In the first edition of this work it was stated that the fever and the characteristic lesions of the disease had been produced in animals by inoculation with this germ. This is now known to be erroneous. As has been stated by FLÜGGE, the essential lesions of typhoid fever may be produced in animals with a number of micro-organisms, among which, however, the Eberth bacillus is not included. The results obtained by FRAENKEL and SIMMONDS and SEITZ have been shown by BEUMER and PEIPER to be fallacious, and the germ with which the experiments were made by VAUGHAN and NOVY, and mentioned in the first edition, is known not to be identical with that of EBERTH.

It is true that this germ induced in dogs a continued fever of from twenty-eight to thirty-five days in duration, terminating

¹ Evidently Flügge is unacquainted with the later researches of Vaughan. See pages 197 *et seq.*

in some instances fatally and revealing ulceration and perforation of the small intestine, but *for this reason* it is known to be different from Eberth's bacillus, because the latter never induces these effects. Notwithstanding this failure to affect the lower animals, the majority of bacteriologists believe, as has been stated, that the Eberth bacillus is the sole and only cause of typhoid fever. In this belief VAUGHAN refuses to concur, and claims that the Eberth bacillus as found in the spleen after death is an involution-form of any one of a number of germs which are found in certain waters. VAUGHAN claims that the typhoid bacillus can be detected in drinking-water by the following characteristics: (1) They grow at 37° , while many of the non-pathogenic germs of water grow only at lower temperatures. (2) They are pathogenic to rats, guinea-pigs, mice, and rabbits. (3) They do not coagulate milk. (4) When grown in milk or gelatin colored blue with litmus, the color is not altered.

In 1885, BRIEGER obtained from pure cultures of the Eberth bacillus a poisonous ptomain, which produced in guinea-pigs a slight flow of saliva, frequency of respiration dilatation of the pupils, profuse diarrhoea, paralysis, and death within from twenty-four to forty-eight hours. Post-mortem examination showed the heart in systole, the lungs hyperæmic, and the intestines contracted and pale. At first BRIEGER was inclined to regard this as the specific poison of typhoid fever, and named it typhotoxin. However, he has more recently modified his opinion and is inclined to regard typhoid fever as due to a mixed infection.

BRIEGER and FRAENKEL have found in the cultures of the Eberth bacillus a proteid which causes death in rabbits after from eight to ten days. They say nothing about the symptoms.

PFEIFFER finds that this typhoid poison is contained in the bacterial cells. From three to four milligrams for each 100 grams of body-weight suffice to kill guinea-pigs. With this poison animals can be rendered immune to the Eberth bacillus, but not to the bacillus coli communis. This typhoid antitoxin is also, according to PFEIFFER, present in the blood of persons convalescing from typhoid fever.

IN 1889 VAUGHAN isolated from mixed cultures from typhoid stools a base, forming crystalline salts and capable of inducing in cats and dogs a marked elevation of temperature accompanied by severe purging. The following is the record of one experiment with this substance: "An aqueous solution of the crystals was given to a dog by the mouth at 3 P.M. The rectal temperature before the administration was 101° F. At 3.15, retching and vomiting set in and continued at intervals for more than two hours. At 3.30, the temperature was 103° F. At 3.55, the animal began to purge. The first discharges contained much fecal matter, but subsequently they were watery and contained mucus plainly stained with blood. At 4, the temperature was 103.5° F., and remained the same at 4.30. The animal was not seen again until 10 A.M. the next day, when its temperature was 100.5°, and recovery seemed complete."

This base was not obtained in quantity sufficient for an ultimate analysis. The platinochlorid crystallizes in fine rhombic prisms and the hydrochlorid in long, delicate, red needles. The red color seems to be inherent to the substance and not due to impurities. The mercury and platinum compounds are insoluble in alcohol, soluble in water. The hydrochlorid is soluble in both water and alcohol.

IN 1890 VAUGHAN reported the isolation, from water supposed to cause typhoid fever, of a number of toxicogenic germs. The chemie products of two of these have been studied. They belong to the proteids, and an analysis of one of them by FREER shows it to belong to the nucleins. These poisons are soluble in water, the opalescent solution showing a distinctly acid reaction. They are not precipitated by heat or nitric acid singly or combined. They dissolve in nitric acid, forming a colorless solution, which becomes yellow on the addition of ammonia. They dissolve in caustic alkalis, and the solution becomes purple on the addition of a dilute solution of copper sulphate.

On white rats these poisons produce symptoms which are identical with those which follow inoculations with the living

germs. The rat seems to shiver with cold and gives evidence of abdominal pain. It lies with its limbs flexed and head drawn down for a few seconds, then stretches out the limbs. It lies on the side for a short time, then sits with the head drawn under the body.

Dogs shiver as with cold, but at the same time the rectal temperature is from one to four degrees above the normal. In some instances vomiting and purging have been induced.

The following experiments seem to show that the poison accumulates in the nerve-centres:

Two guinea-pigs were treated with hypodermic injections of one of these poisons, the amount used being about ten times the dose which ordinarily proves fatal to these animals. Within twelve hours both were dead. Plate cultures made from the liver, spleen, blood, brain, and spinal cord remained sterile. Small quantities of the brain and spinal cord were rubbed up in a sterilized dish with sterilized water, and 2 c.c. of the emulsion were injected under the skin of each of four guinea-pigs. These animals seemed to be very excitable the next day, throwing themselves about violently in the cages when slight noises were made near them. Within a period of from sixteen to twenty-four days all died. This experiment needs repetition, and it will be necessary to prepare and inject similar emulsions made from other organs before any positive conclusions can be drawn.

In a study of fatal cases of typhoid fever at Bucharest BABES finds that the typical germ differs markedly from that of Eberth.

SWINE-PLAGUE, OR HOG-CHOLERA. — The researches of LOEFFLER, SCHÜTZ, LYDTIN, and SCHOTTELIUS, in Europe, and of BILLINGS and SALMON, in this country, have demonstrated the existence among swine of at least three infectious diseases. These are—

1. Hog-erysipelas, or rouget of France, or Schweinerothlauf of Germany.

2. German swine-plague, or Schweineseuche.

3. American swine-plague (BILLINGS), or hog-cholera (SALMON).

The first two of these are exclusively European diseases, and their chemie poisons have not been studied.

The American swine-plague is præminently a disease of the digestive tract involving most markedly the large intestine. It is the great swine disease of this country, and is probably present in England, where it is associated with other diseases under the name of swine fever. A disease which was observed in Denmark and Sweden for the first time in 1888-89, and known as swine-pest or swine-diphtheria, has been shown by SELANDER and FROSCHE and others to be identical with our swine-plague. In the summer of 1889 France was visited by a swine disease, which is considered by CORNIL and CHANTE-MESSE to be identical with the German swine-plague, but which RIETSCHE and JOBERT, after a comparative study of the micro-organisms, pronounce as the American disease. In this country we have at present no positive demonstration of the existence of any other infectious swine disease. The swine-plague of SALMON has been the subject of considerable discussion, but its existence as a distinct epidemic disease can hardly be said to be established.

The following statements concerning the chemie poisons refer to the swine-plague of BILLINGS or the hog-cholera of SALMON, which are only two names for one disease.

In pure cultures of this bacillus NOVY has found a poisonous base, which probably has the composition $C_{10}H_{26}N_2$, and to which he has provisionally given the name susotoxin. One hundred milligrams of the hydrochlorid of this base cause in white rats convulsive tremors and death within one and one-half hours. Post-mortem examination shows the heart in diastole, lungs pale, stomach contracted, a serous effusion in the thoracic cavity, and the subcutaneous tissue pale and oedematous.

NOVY has also obtained a poisonous proteid from cultures of this germ. The following experiments illustrate the effects obtained with this body: 100, 50, and 25 milligrams, respec-

tively, were injected into three young rats from the same litter. The animal which received 100 milligrams soon began to crawl about on its belly, being unable to rise. The eyes were soon filled with a thick secretion and the toes became red. Finally it became quiet, lying on its belly, with feet extended. The respirations became deeper, and a coma-like condition set in. The animal died, without convulsions, within about three hours. The rat which received 50 milligrams went through the same course of symptoms, but these were less intense. Death resulted four hours after the injection. The one which received 25 milligrams became very sick, but finally recovered, and one week later it was given another injection of 30 milligrams, which produced scarcely any effect. Then it was treated at intervals of five, three, five, two, and four days, respectively, to 40, 50, 75, 100, and 125 milligrams without effect. Three days after the last injection the animal was inoculated with 1 cubic centigram of a bouillon culture of the highly virulent germ. Only a slight temporary effect was observed during the first day, after which recovery was complete and permanent. A control-rat which was given the same quantity of the culture sickened the next day and died one week later. From this it will be seen that the animal was rendered immune against the disease.

SCHWEINITZ also reports the detection of a slightly poisonous base, which he designates as sucholotoxin, and a poisonous proteid, and with these he has been able to secure immunity in guinea-pigs against the virulent germ. The proteid body is classed among the albumoses, and is said to crystallize in white, translucent plates when dried *in vacuo* over sulphuric acid and to form needle-like crystals with platinum chlorid. No one else has reported a crystalline bacterial proteid, and this body is deserving of a more extended study.

SCHWEINITZ has also reported the isolation of soluble ferments, or enzymes, from cultures of the hog-cholera germ. From cultures in milk both peptonizing and diastatic ferments were obtained. These were destroyed by heating above 55°; they contain nitrogen, and, when pure, do not give the albu-

minoid reactions. Injections of the soluble ferments conferred immunity.

As stated above, immunity can be imparted to hog-cholera by injection of toxic products and by ferments. In 1892 NOVY obtained immunity in rabbits by injection of blood-serum of immunized animals. Similar results were obtained by SCHWEINITZ. In the same year METSCHNIKOFF published his studies on immunization with blood-serum of rabbits immune to hog-cholera. According to SMITH and MOORE, the latter did not work with the hog-cholera germ, but with that of swine-plague. These investigators obtained only partial immunity with blood-serum of immune guinea-pigs, and none with that of rabbits.

RABBIT SEPTICEMIA.—HOFFA has killed rabbits by inoculation with pure cultures of the bacillus of this disease, and has isolated from the bodies of these animals methylguanidin, while in the bodies of healthy rabbits this poison could not be found. The fatal dose of methylguanidin for rabbits was found to be 0.2 gram when given subcutaneously. Since HUEPPE has suggested that the bacterium of chicken-cholera is identical with that of rabbit septicemia, chickens were poisoned with methylguanidin, and the symptoms were observed to be analogous to those of the disease.

PNEUMONIA.—BONARDI has made a chemie study of the diplococcus of FRAENKEL. He finds certain poisons—ptomaines—which he has been unable as yet to obtain in quantity sufficient for ultimate analysis. He also claims to have secured immunity against the germ by treating rabbits with small quantities of the chemie poisons.

MALIGNANT OEDEMA.—KERRY finds that the bacillus of this disease decomposes albumin with the formation of fatty acids, leucin, hydro-paracumaric acid, and a foul-smelling oil of the composition $C_{24}H_{46}O_4$. This oil is insoluble in water, alkalis, and acids, easily soluble in ether, benzol, bisulphide

of carbon, and alcohol. It is optically inactive, and on being oxidized furnishes valerianic acid. Nothing is said concerning its action upon animals. Among the gaseous products are carbonic acid, hydrogen, and marsh gas. The author was unable to determine whether or not free nitrogen is formed.

PUERPERAL FEVER.—BOURGET claims to have isolated several ptomaines from the urine of women with puerperal fever. His conclusions are as follows: (1) In puerperal fever the urine contains highly poisonous bases. (2) The toxicity of the urine is most marked when the symptoms of the disease are most grave, and diminishes as the symptoms abate. (3) The ptomaines obtained from the urine prove fatal when injected into frogs and guinea-pigs. (4) Toxic bases, resembling those obtained from the urine, were extracted from the viscera of a woman who had died of puerperal fever.

GLANDERS.—The poison of this disease is contained in the bacterial cell, and is known as mallein or morvine. Sterilized cultures of the glanders bacillus containing this chemic poison are now used for the purpose of diagnosing the disease in horses in the same way that tuberculin is employed to detect tuberculosis in cows. In glandered horses the subcutaneous injection of small quantities of mallein causes a more marked elevation of temperature than it does in healthy horses. There is nothing specific, however, as has been shown by the researches of SCHATTENFROH, in this action of mallein. The same effect is produced in glandered horses by the injection of toxins obtained from other bacteria.

Commercial mallein is prepared in a number of ways, one of which is the following:

Growths of the glanders bacillus, from ten to fourteen days old, on potatoes are removed with a sterilized spatula and rubbed up with sterilized water in the proportion of one part of the moist bacilli to nine parts of water. This emulsion is allowed to stand for twenty-four hours, and then heated to 110° for fifteen minutes; next it is filtered through porcelain,

thirty per cent. of glycerin added, and concentrated at low temperature on the water-bath to one-eighth of the original volume. This is again sterilized at 110°. The preparation is now ready for use and consists of a clear, yellowish, odorless fluid of feebly acid or neutral reaction.

KRESSLING found in mallein prepared by the above-given method peptons, globulins, xanthin, guanin, small quantities of tyrosin and leucin, and traces of volatile fatty acids and ammonia.

BONOME fails to find any direct relation between the susceptibility of an animal to glanders and the reaction produced in the same species by mallein. Thus, dogs and guinea-pigs are easily infected with glanders, while the chemie poison has but little effect on these animals. He reports a case of glanders in man apparently cured by the continued use of small doses of mallein.

MASTITIS BOVIS.—At the request of the Swiss Minister of Agriculture, several investigators undertook the study of inflammation of the udders of cows and goats. GUILLEBEAU, who did the bacteriological work, found a number of micro-organisms present. NENCKI has studied the chemie products of the streptococcus mastidis sporadica, the bacillus Guillebeau, and bacillus Guillebeau c.

The first produces in a solution containing pepton, salt, grape-sugar, and calcium carbonate, traces of a volatile iodo-form-forming substance, of volatile fatty acids and larger quantities of paralactic acid, and a large amount of carbonic acid gas.

The second, which is the most frequent cause of mastitis, and which is said to be the cause of puffy cheese, produced ethylic alcohol, paralactic acid, carbonic acid gas, acetic acid, and hydrogen.

The third produced essentially the same substances as the second.

NENCKI thinks that these germs decompose the milk in the gland and the acids formed set up the inflammation.

CHAPTER VII.

THE GERMICIDAL CONSTITUENT OF BLOOD-SERUM.

As early as 1872 LEWIS and D. CUNNINGHAM demonstrated the fact that bacteria injected into the circulation rapidly disappear. In the blood of twelve animals that had been treated with such injections bacteria could be found after six hours in only seven. Of thirty animals, bacteria were found after twenty-four hours in the blood of only fourteen, and of seventeen animals, bacteria were found in only two when the examination was made from two to seven days after the injection.

In 1874 TRAUBE and GSCHIEDLEN found that arterial blood, taken under antiseptic precautions from a rabbit into the jugular vein of which one and one-half c.c. of a fluid rich in putrefactive germs had been injected forty-eight hours previously, failed to undergo decomposition for months. These investigators attributed the germicidal properties of the blood to its ozonized oxygen. Similar results were obtained by FODOR and by WYSSOKOWICZ. The latter accounted for the disappearance of the germs, not by supposing that they were destroyed by the blood, but that they found lodgement in the capillaries.

The first experiments made with extra-vascular blood were conducted by GROHMANN under the direction of A. Schmidt in his researches upon the cause of coagulation. It was found that anthrax bacilli, after being kept in plasma, were less virulent, as was demonstrated by their effect upon rabbits. GROHMANN supposed that in some way the bacteria were influenced by the process of coagulation.

In 1887 FODOR made a second contribution to this subject, and in this he combated the retention-theory of WYSSOKOWICZ. One minute after the injection of one c.c. of anthrax culture

into the jugular vein, in eight samples of blood, FODOR found only one colony of the bacillus. Then he took the blood from the heart with a sterilized pipette and added anthrax bacilli to it. This was kept at 38° , and plates made from time to time showed a rapid diminution in the number of germs; after a time, when the blood had lost its germicidal properties, the number of germs began to increase.

In 1888, NUTTALL, working under the direction of Flügge, used defibrinated blood taken from various species of animals, rabbits, mice, pigeons, and sheep, and found that this blood destroyed the bacillus anthracis, bacillus subtilis, bacillus megaterium, and staphylococcus pyogenes aureus, when brought in contact with them. He also confirmed the further finding of FODOR that after a while the blood loses its germicidal properties and becomes a suitable culture-medium in which the germs grow abundantly.

NISSEN continued this work under Flügge's direction and reached the following conclusions:

1. The addition of small quantities of sterilized salt-solution or bouillon to the blood does not destroy its germicidal properties.

2. Cholera-germs and Eberth's bacilli are easily destroyed by fresh blood.

3. For a given volume of blood there is a maximum amount of bacilli which can be destroyed.

4. Blood the coagulability of which has been destroyed by the injection of pepton is still germicidal.

5. Blood in which coagulation is prevented by the addition of 25 per cent. of magnesium sulphate has its germicidal properties decreased.

6. Filtered blood-plasma from the horse is germicidal.

BEHRING has attributed the action of the blood of the white rat on anthrax bacilli to its great alkalinity. He has made a number of titrations, by which he shows that the blood-serum of the rat is somewhat more alkaline than that of certain animals that are more susceptible to anthrax, such as the rabbit, the guinea-pig, and the cow. His deduction is not

justified, because there are many other and more important points in which these animals differ more markedly from the white rat than in slight differences in the alkalinity of the blood-serum. Had he shown that the blood of the adult rat, which is not susceptible to anthrax, is more alkaline than that of the young rat, which is susceptible, his argument would have been more plausible; but even then it would not have deserved the dignity of positive evidence.

In 1890, BUCHNER, aided by Voit, Sittman, and Orthenberger, made a most valuable contribution to our knowledge of the germicidal properties of blood. The results of this work are stated as follows:

1. The germicidal action of blood is not due to phagocytes, because it is not influenced by the alternate freezing and thawing of the blood, by which the leucocytes of the rabbit are destroyed.

2. The germicidal properties of the cell-free serum must be due to its soluble constituents.

3. Neither neutralization of the serum, nor the addition of pepsin, nor the removal of carbon dioxid gas, nor treatment with oxygen has any effect upon the germicidal properties of the blood.

4. Dialysis of the serum against water destroys its activity, while dialysis against 0.75 per cent. salt solution does not. In the diffusate there is no germicidal substance. The loss by dialysis with water must be due to the withdrawal of the inorganic salts of the serum.

5. The same is shown to be the case when the serum is diluted with water and when it is diluted with the salt solution. In the former instance the germicidal action is destroyed, while in the latter it is not.

6. The inorganic salts have in and of themselves no germicidal action. They are active only in so far as they affect the normal properties of the albuminates of the serum. The germicidal properties of the serum reside in its albuminous constituents.

7. The difference in the effects of the active serum and that

which has been heated to 55° is due to the altered condition of the albuminate. The difference may possibly be a chemie one (due to changes within the molecule) or it may be due to alterations in mycelial structure (micellaren Bau). The albuminous bodies act upon the bacteria only when the former are in an active state.

We wish at this point to call attention to an inconsistency between the results obtained by BUCHNER and the conclusions that he draws. In experiment No. 45 he renders the serum slightly acid and adds 0.1 gram of pepsin to each 5 c.c. of serum (showing by a side experiment that this pepsin actively digests coagulated egg-albumin in neutral solution) and finds that the digestive action of the pepsin does not lessen the germicidal properties of the serum. In fact he states this in his conclusions, but his ultimate opinion, and the one held by him in his latest contribution, is that the germicidal constituent of the blood is the serum-albumin. How much serum-albumin remains in blood-serum after it has been thoroughly digested with pepsin? He could scarcely have chosen a more positive method of demonstrating that the germicidal constituent is not serum-albumin. Either his pepsin was not active, and on this supposition his experiment is without value, or the active constituent of blood-serum is a substance that is not destroyed or materially altered by peptic digestion. We know that the peptons not only have no germicidal properties, but that they belong to that class of proteids that is most favorable to the growth and development of germs. We recognize this fact when we add peptons to the various artificial media of which we cultivate germs. However, we shall return to this subject. At present we shall proceed with the literature of the subject.

The successful researches of BUCHNER led many other investigators to enter this field of experimentation, and some of them have made valuable contributions to our knowledge of the germicidal action of the blood under varying conditions, but so far as the nature of the germicidal constituent is concerned but little or no progress has been made. PRUDEN

found that ascitic and hydrocele fluids restrain the development of certain germs. ROVIGNI reported that the germicidal action of the blood is increased in febrile conditions. PEKELIARING enclosed anthrax-spores in bits of parchment and introduced them under the skin of rabbits. Thus treated, the spores soon lost their virulence and finally their capability of growth. The destruction of these spores could not have been due to phagocytes, which did not penetrate the parchment, but must have been caused by soluble poisons. BEHRING and NISSEN found that the serum of the white rat, the dog, and the rabbit destroys anthrax bacilli, while serum obtained from the mouse, sheep, guinea-pig, chicken, pigeon, and frog, has no such action. It will be observed from this that there is no constant relation between the germicidal action of the blood of animals of different species and their susceptibility to the disease caused by the germ. Thus, the rabbit is highly susceptible to anthrax, notwithstanding the fact that its blood destroys large numbers of these germs. On the other hand, the chicken is immune to anthrax from the moment when it comes from the shell, and yet the bacillus anthracis grows luxuriantly in the extra-vascular blood of the chick. This demonstrates that there is a great difference between the action of extra-vascular blood and that circulating in the body, constantly fed, and, in case of need, altered in composition by certain glands.

HALLIBURTON has prepared from the lymphatic glands a globulin which he designates as cell-globulin β , and which agrees with fibrin-ferment in inducing coagulation in plasma. HANKIN has tested the germicidal properties of this cell-globulin. His experiments have been conducted in the following manner: The lymphatic glands (in later experiments the spleen also) of a dog, or of a cat, are freed as much as possible from fat and connective tissue, then finally divided and extracted with dilute solution of sodium sulphate (one part of a saturated solution to nine parts of water). The cell-globulin passes into solution, while the other proteids are but sparingly soluble. After twenty-four hours the fluid is filtered

and mixed with an excess of alcohol. The voluminous precipitate containing the cell-globulin is collected on a filter and washed with absolute alcohol. For use, a part is dissolved in water, and a small quantity of a bouillon-culture of the anthrax bacillus is added. From time to time plate-cultures are made, along with control-plates, and in this way the germicidal properties of the substance are demonstrated. HANKIN closes this contribution with the following conclusions:

1. HALLIBURTON'S cell-globulin β has marked germicidal properties.

2. In this respect it differs from fibrin-ferment.

3. The germicidal properties of this substance seem to be identical with those of serum as described by BUCHNER, NISSEN, and NUTTALL.

4. The active properties of the serum are probably due to this or to an allied body.

BITTER has repeated the experiments of HANKIN, but fails to confirm them. BITTER states that he has followed HANKIN'S directions exactly. However this may be, it is certain that the spleen contains a germicidal substance, but whether it can be extracted by the method of HANKIN or not we do not know. That the germicidal constituent of the spleen is identical with HALLIBURTON'S cell-globulin β or with any other globulin we very much doubt. It certainly is a nuclein, and it is altogether possible that HANKIN obtained traces of this nuclein in his extracts. In this case the extract would show, or fail to show, germicidal properties according to the relative amounts of nuclein and other substances present. The less globulin and the more nuclein present the more marked would the germicidal effect be.

CHRISTMAS has prepared a germicidal substance from the spleen and other organs by the following method:

The animal is killed with ether, opened under antiseptic precautions and the organ removed, cut into fine pieces, covered with 50 cubic centimetres of glycerin and allowed to stand for twenty-four hours, and then filtered. The filtrate is precipitated with five times its volume of alcohol, and this

fluid is immediately decanted. The precipitate is washed with absolute alcohol in order to remove the glycerin. Then the traces of alcohol are removed by pressure and the precipitate dissolved in 25 cubic centimetres of distilled water. Through this solution air is driven for some hours in order to remove the traces of alcohol. Then the fluid is filtered and its germicidal action tested.

BITTER has also examined this method, and the impartial reader must see that he has not done so with fairness. However, this fact renders the work all the more valuable because his results confirm the statements of CHRISTMAS. BITTER killed his animals by venesection, and, in some cases at least, prepared the substance in unsterilized vessels; but even when this was done the solution was germ-free and manifested marked germicidal properties. BITTER finally finds a difference between this substance and the germicidal constituent of blood-serum; the latter, he states, is certainly destroyed by a temperature of 65° , while the solution of CHRISTMAS, after having been heated to this temperature, is still capable of destroying from 35,000 to 40,000 typhoid bacilli within four hours. BUCHNER, in his latest contribution to the subject, has the following to say in condemnation of CHRISTMAS:

“A method given by CHRISTMAS for the preparation of germicidal solutions from the organs of normal rabbits has also been tested by BITTER. Germicidal solutions were indeed obtained, which, however, differed materially from active serum, for in three experiments, notwithstanding heating to 65° , the germicidal action remained.”

It is altogether possible that the more powerful action of the solution made by CHRISTMAS is due to the fact that it contained the germicidal substances in more nearly a chemically pure condition than they exist in blood-serum. It is also highly probable that the arrest of the germicidal activity of blood-serum by a temperature of 55° is not due to the destruction of its germicidal constituent, but is due to the action of the heat on other constituents of the fluid.

Some attempts have been made to determine the nature or

the germicidal constituent by the action of precipitating reagents on the proteids of blood-serum. In his latest contribution, BUCHNER states that he has not been able to obtain a germicidal solution by precipitating all the proteids with absolute alcohol, freeing the precipitate from alcohol, drying it, and then redissolving. He does not give the methods employed in freeing the precipitate from alcohol, the temperature or the conditions under which it was dried, or the nature of the menstruum by which resolution was effected. In the absence of these needed details his conclusion that alcohol destroys the germicidal substance must remain open to question. On the other hand, CHRISTMAS states that when the proteids are precipitated with alcohol, and the precipitate dissolved in a volume of water equal to that of the original serum, the solution thus obtained has a more powerful germicidal action than the serum. BITTER in an experimental review of the statement of CHRISTMAS gives the following detailed account of one experiment :

“Ten cubic centimetres of serum were poured into 50 cubic centimetres of alcohol (strength of alcohol not given), stirred, and the precipitate immediately separated from the alcohol by filtration. (He fails to state whether or not sterilized filter-paper was used.) The precipitate was freed from alcohol by pressure between folds of filter-paper (again he fails to state whether or not this paper was sterilized), then dried at 37°, and mixed with 10 cubic centimetres of sterilized distilled water. On being allowed to stand for a short time at 37°, nearly all of the precipitate was redissolved. The solution was then separated from the deposit by filtration (through unsterilized filter paper?) and tested.”

It can scarcely be a matter of surprise that BITTER found germs nearly always present in the solution obtained in this careless manner. However, he did find that the germs present did not develop when the solution was kept at 37°, and, moreover, that germs added to this solution were destroyed. BITTER concludes that in truth anthrax and typhoid bacilli

are destroyed by "precipitated serum," but not so energetically as by normal serum.

EMMERICH, TSUBOI, STEINMETZ, and LÖW have made interesting and valuable contributions relating to the effect of precipitation of the proteids upon the germicidal action of blood-serum. An active serum was dialyzed in a sterilized parchment-paper tube against water for from twelve to eighteen hours. By the expiration of that time the serum-globulin, becoming insoluble on account of the withdrawal of inorganic salts, was deposited. The dialyzer was dried with sterilized filter-paper and the globulin-free serum was precipitated with several volumes of alcohol. The precipitate was collected on a sterilized "falten-filter" and the alcohol removed from the precipitate by sterilized porous plates and filter-paper. The precipitate was then finely divided, dried for half an hour in vacuo at 36° , then rubbed up in a sterilized mortar and dissolved in sterilized water, to which salt-solution had been added. In the solution thus prepared germs did not show, after from three to four hours, either a marked increase or decrease, but when the solution was heated to 100° , allowed to cool, and then inoculated with germs, the increase was four hundred fold within four hours. It was next found that if instead of water a 0.05 per cent. aqueous solution of potassic hydrate was employed in dissolving the alcoholic precipitate in the globulin-free serum, this solution possessed all the germicidal strength of the original serum. The same was found to be true of dilute alkaline solutions of the alcohol precipitate in serum from which the globulin had not been removed. The dilute alkali was shown not to have any germicidal action in and of itself. From these experiments the investigators mentioned conclude that the germicidal constituent of blood-serum is an alkaline compound of serum-albumin. They also found that heating the serum-albumin alkaline solution to 65° , or higher, destroyed its germicidal action, and they explain this effect of heat on blood-serum and on their artificial solution by supposing that the high temperature breaks up the combination of the alkali

with the serum-albumin. Furthermore, they found that a serum that had been rendered inactive by a temperature of 55° could be regenerated, in part at least, by the addition of the small amount of alkali mentioned.

Since FODOR and ZUNTZ have shown that freshly drawn blood rapidly decreases in alkalinity on standing *in vitro*, an explanation of the fact that blood-serum rapidly loses its germicidal properties naturally suggests itself. EMMERICH and his coworkers confirm their belief in this theory by demonstrating that blood-serum that has been rendered very feebly acid (0.67 part of sulphuric acid per mille) has no germicidal action, but furnishes a good culture-medium.

The foregoing investigations are very valuable, inasmuch as they show the important rôle that the small amount of alkali plays in the germicidal action of blood-serum. This had, indeed, already been demonstrated by FODOR by quite a different line of investigation. This experimenter found that the resistance of rabbits to anthrax is markedly increased by the administration, by the stomach or subcutaneously, of sodium phosphate, carbonate, or bicarbonate, or of potassium carbonate.

Löw concludes that the introduction of the alkali into the albumin-molecule increases its lability, and he cites examples from organic chemistry in support of this view.

There are some additional points of interest in the theory of EMMERICH and his assistants. As has been stated, they believe that the serum-albumin is the germicide, but they think it highly probable that only a comparatively small part of the albumin is active, and this small part, they suppose, originates in the albumin of the daily food, which is converted into lymph-cells, and by the disintegration of these it passes into solution in the blood. They admit, however, that there are some reasons for believing, with BUCHNER, that the whole of the serum-albumin is active. They state that it is possible, *but highly improbable*, that the germicidal substance is not the serum-albumin, but some substance that is precipitated along with this by alcohol and other agents.

We hope to show that the germicidal agent is not serum-albumin, and that this "highly improbable" substance does exist.

In a short and somewhat unsatisfactory review of the report of Emmerich and his coworkers, BUCHNER devotes himself to a consideration of the question of the regeneration of serum rendered inactive by heating to 55° on the addition of an alkali. He details one experiment made by himself on this point. The experiment confirms the work of Emmerich, but BUCHNER offers an interpretation that is wholly theoretic, and by no means convincing. He finds that the regenerated serum, when heated to 60° , still has a retarding effect upon the growth of germs, and he argues from this that the germicidal action of the "regenerated serum" is (for some unknown reason) due to its being less suited to the growth of bacteria. No one knows better than BUCHNER the influence of various chemie substances on the temperature at which an active serum is converted into an inactive form, and yet he overlooks altogether the possible effect of increased alkalinity on this conversion. Had he heated the regenerated serum to 100° he would then have found that it forms a very fertile culture-medium.

HANKIN has recently published a paper that is more valuable in its suggestions than in its experimental details. He suggests that the germicidal substance is a special secretion of the eosinophile granular cells. The granular matter in these cells is, according to his theory, the antecedent of the germicidal substance.

In 1893 VAUGHAN and MCCLINTOCK, after reviewing the literature as just stated, report their work on the germicidal constituent of blood-serum as follows:

1. The serum-albumin is not the germicidal substance in blood-serum. Either this must be true, or the experiment by which BUCHNER demonstrated that an active pepsin does not destroy the germicidal action of blood-serum must have been an error; because peptic digestion readily and completely

converts serum-albumin into peptons, and we know that peptons are especially favorable to bacterial growth.

2. The germicidal substance must belong to the proteids. Otherwise it would be difficult to explain the fact that a temperature of 55° renders blood-serum inactive.

3. The only proteid likely to be present in blood-serum and which is not destroyed by peptic digestion is nuclein.

Having reached these conclusions, the following questions naturally present themselves :

1. Is there a nuclein in blood-serum ?

2. Has the nuclein, if there be one, germicidal properties ?

These questions we have attempted to answer.

Dogs and rabbits were the animals from which the serum was obtained. Healthy animals that had not previously undergone any experimentation were selected. The animal was firmly fixed in a holder, the carotid was laid bare under antiseptic precautions. A ligature and a small clamp were applied to the artery about two inches apart, the former distad and the latter centrad. Then a slit in the artery was made with a sterilized knife, and a small sterilized glass canula, with sterilized and dried rubber tube leading into a sterilized Erlenmayer flask, was introduced into the artery and held in place by another ligature. Then the clamp was removed and the blood flowed into the flask. In each case the animal was bled to death. The flask containing the blood was placed in the ice-chest, and allowed to remain for twenty-four hours. By the expiration of this time a wine-colored serum had separated. This serum was poured into a second sterilized flask, and about ten volumes of a mixture of equal parts of absolute alcohol and ether were added. This produced a voluminous precipitate that was nearly white. This was allowed to stand twenty-four hours, and in some cases much longer, the alcohol and ether twice, or more often, during the time, being decanted and replaced by equal volumes. Then the supernatant fluid was decanted, and an equal volume of a 0.2 per cent. solution of hydrochloric acid con-

taining active pepsin was added, and the flask placed in an incubator at 38° , and the digestion was continued until the fluid failed to respond to the biuret-test for peptons. Each time this test was made the fluid was decanted from the undigested portion and replaced by an equal volume of fresh digestive fluid. In some instances the flask containing this fluid was allowed to stand in the incubator for several days. This was not necessary in order to complete the digestion, but was allowed as a matter of convenience. In all cases the digestion was prompt, and proceeded to a certain point, when it ceased altogether. The undigested portion was small in amount and grayish in color. This was collected on a small sterilized filter, and washed first with 0.2 per cent. solution of hydrochloric acid, and then with alcohol. After the washing with alcohol the filter was allowed to stand exposed to the air for half an hour or longer in order that all of the alcohol might pass through or evaporate. The precipitate was then dissolved in a sterilized solution of potassic hydrate. The strength of this alkaline solution usually employed was 0.12 per cent. Usually this solution contained in addition to the alkali 0.6 per cent. of sodium chlorid. In some instances a solution containing 1.2 grams of potassic hydrate, 6 grams of sodium chlorid, and 1 gram each of sodium bicarbonate and disodium hydrogen phosphate to one litre of water was employed as a solvent. The solution was filtered through a Chamberland tube and received in a sterilized flask.

The solution thus obtained was perfectly clear, colorless, and did not respond to the biuret-test. The addition of strong nitric acid produced a cloudiness, which dissolved on the further addition of the acid. This acid solution did not become yellow on being heated, but did so after the addition of ammonia.

We have now answered the first question. Blood-serum contains a nuclein. We hope to investigate, at some time in the future, the relation between this nuclein and fibrin-ferment.

The origin of the nuclein found now for the first time in

blood-serum is an interesting question. Does it come from the disintegration of the polynuclear cells, or shall we regard certain white blood-corpuscles as unicellular organs whose function it is to secrete this nuclein?

In proceeding to determine whether or not this nuclein has germicidal properties, the solution was distributed in sterilized test-tubes, five c.c. being placed in each tube. It should be stated that in dissolving the nuclein the volume of the solvent employed was in all cases the same as that of the blood-serum from which the nuclein was obtained. These tubes were inoculated with different germs and plates made at varying intervals of time, in order to test the germicidal action. One and the same platinum loop was used in the preparation of each plate.

EXPERIMENT I.

A nuclein-tube was inoculated with the bacillus of *Asiatic cholera*, and plates made from this gave the following results:

Time, No. of colonies, }	Imme- diately	5 min.	15 min.	30 min.	1 hr.	1½ hr.	22 hrs.
	2100	43	54	71	90	115	1200

That the alkali in which this nuclein was dissolved did not cause the decrease in the number of germs is shown by the subsequent increase.

EXPERIMENT II.—*Staphylococcus pyogenes aureus*.

Time, No. of colonies,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
	4000	1720	1050	810	0

EXPERIMENT III.—*Anthrax-bacillus without spores*.

Time, No. of colonies,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
	100	43	10	1	0

EXPERIMENT IV.—*Cholera-germ*.

Time, No. of colonies,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
	470	45	1	0	410

It may be stated that the final increase in the number of cholera-germs occurred both in the nuclein-solution prepared from the serum of the rabbit and that prepared from the serum of the dog.

EXPERIMENT V.—*Staphylococcus pyogenes aureus*.

Time,	Immediately	1 hr.	5 hrs.	19 hrs.	24 hrs.
No. of colonies,	Countless	22,000	12,525	155	0

EXPERIMENT VI.—*Anthrax-bacillus without spores*.

Time,	Immediately	1 hr.	5 hrs.	19 hrs.	24 hrs.
No. of colonies,	1120	165	0	0	0

All of the foregoing experiments were made with the solution of nuclein in sterilized water containing 0.12 per cent. potassic hydrate and 0.6 per cent. of sodium chlorid. The following were made in the other solution mentioned. It may be stated that the culture of the aureus experimented with retained its vitality for days in water containing 0.5 per cent. of potassic hydrate :

EXPERIMENT VII.—*Staphylococcus pyogenes aureus*.

Time,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
No. of colonies,	5000	2500	1600	1200	0

EXPERIMENT VIII.—*Anthrax-bacillus without spores*.

Time,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
No. of colonies,	43	7	0	0	0

EXPERIMENT IX.—*Cholera-bacillus*.

Time,	Immediately	1 hr.	4 hrs.	7 hrs.	24 hrs.
No. of colonies,	350	105	150	42	0

EXPERIMENT X.—*Staphylococcus pyogenes aureus*.

Time,	Immediately	1 hr.	5 hrs.	19 hrs.	24 hrs.
No. of colonies,	Countless	25,000	5525	65	500

EXPERIMENT XI.—*Anthrax-bacillus without spores*.

Time,	Immediately	1 hr.	5 hrs.	19 hrs.	24 hrs.
No. of colonies,	430	0	0	0	0

We have made many other tests of the germicidal action of the nuclein obtained from blood-serum, but as all of them gave practically the same results further repetition is unnecessary.

HANX criticises the evidence, given in this chapter, that blood-serum contains nuclein, stating that the portion of the alcoholic precipitate undissolved by the pepsin and hydrochloric acid might have been fibrin or albumin. This criti-

cism is much strained for the following reasons: (1) The precipitate produced on the addition of alcohol to blood-serum does not contain fibrin. (2) Fibrin would not be so readily soluble in the very dilute alkali used. (3) Fibrin or albumin dissolved in alkali would not fail to give the biuret and xanthoproteic tests, nor would such solutions have any germicidal action. However, the presence of nucleinic acid in blood-serum has been confirmed by the work of LILIENTHAL, whose paper was published in 1895, while that of the American investigators, as given in this chapter, was published in 1893.

CHAPTER VIII.

IMMUNITY, ANTITOXINS, AND SERUM-THERAPY.

THE subject of immunity is one of the most interesting, and at the same time one of the most perplexing problems with which the student of scientific medicine can busy himself. To render man immune to such deadly infectious diseases as tetanus, diphtheria, cholera, typhoid fever, and tuberculosis is the task that now occupies the time and energy of many honest toilers in the laboratories of science. The brilliant success of JENNER with vaccination against smallpox and the great achievement of PASTEUR in hydrophobia have given ground for the hope that like results may be secured in protecting man from other diseases. Scores of busy workers have discovered many facts, some of which must ultimately prove of benefit to mankind. The deduction of general principles and the establishment of fixed laws seem, as yet, however, quite impracticable. Among these discovered facts there are many apparent contradictions, and those which have been found to be true in the study of one disease in a given species of animals are found to be false when applied to another disease, or to the same disease as it manifests itself in other animals. For the present we are compelled to content ourselves with a statement of the most important and trustworthy of the announced discoveries, trusting that the labors of the future will unravel the many mysteries which the knowledge of the present cannot solve. It will be convenient to discuss the subject under the two forms of natural and acquired immunity.

NATURAL IMMUNITY.—Some animals are immune by nature to certain infectious diseases. The chick from the moment when it breaks through the shell is immune to anthrax.

This disease is common among cattle and sheep, and is easily transmitted to mice, rabbits, and guinea-pigs by inoculation, while the carnivora in general and birds are immune. However, immunity to anthrax is not altogether, at least, limited by those lines which differentiate animals into species; thus, while ordinary sheep are very susceptible to this disease, and the annual loss in Europe among these animals from this scourge is great, Algerian sheep are quite immune. The lower animals are in nature practically immune to cholera, typhoid and typhus fevers, relapsing fever, scarlet fever, diphtheria, and influenza. On the other hand, man is exempt from certain septicæmias that manifest themselves among one or more species of the lower animals, such as hog-cholera, chicken-cholera, swine-erysipelas, etc. Some of the infectious diseases are found most frequently among the members of a given species, but are occasionally transmitted to others. Thus, glanders, although essentially a disease of horses, may be transmitted to man and certain other animals. Other infectious diseases, as tuberculosis and tetanus, are common to the most diverse species.

It is important to note the fact that certain animals may be very susceptible to a given infection and yet be free from it in nature. Probably of all animals the guinea-pig is the most susceptible to tuberculosis, and yet this animal practically never develops this disease unless it be inoculated. In the more than two thousand guinea-pigs and rabbits used by CORNET, only four spontaneously developed tuberculosis. Both rabbits and guinea-pigs are highly susceptible to anthrax, and yet no one has ever observed the spontaneous development of this disease in either of these animals. These animals are free from these diseases except when inoculated, because they are not exposed to the infection in nature. Their freedom is not due to the possession of any immunity, but to the absence of exposure. Indeed, it is highly probable that their marked susceptibility is due to the fact that for successive generations they have not been exposed to the infection. People of the North are free from yellow fever at home, but

let them visit the South and expose themselves to the infection, and their susceptibility is much greater than those who have always lived in the South. The freedom of the Canadian at home from yellow fever is not due to immunity, but to absence of exposure. Instead of possessing immunity he is highly susceptible, and this high degree of susceptibility is probably due to the fact that neither he nor his ancestors have been exposed to this infection. Instances of the great susceptibility of races and nations when first visited by infectious diseases are common in the history of epidemics. Small-pox brought to the Western Hemisphere by the Spanish discoverers soon destroyed entire tribes of Indians, and the fearful mortality from the epidemic of measles along the Amazon in 1749 was due, at least partially, to the fact that the inhabitants were especially susceptible to a new infection. Syphilis proved so disastrous to the people of the South Pacific Islands for the same reason. Exposure to a given disease for successive generations is one of the factors in the evolution of natural immunity. We do not claim that this is an essential factor, nor will we attempt to estimate its relative importance, but will content ourselves with naming it as a factor.

Immunity is often at least only a diminished susceptibility. Absolute immunity would be a condition in which no quantity of the infecting agent, however virulent, could do the organism the slightest harm. Such immunity probably exists, but its possession is rare among the higher animals. The adult rat is practically immune to anthrax, but it succumbs to large injections of the virulent germ, or small doses prove fatal, provided that the rat has been reduced in vitality by exhaustive exercise before the inoculation is made. As has been stated, the chick is naturally immune to anthrax, but it can be rendered susceptible to this disease by artificially reducing the body-temperature. Mice in health are not susceptible to glanders, but susceptibility to the bacillus may be established by rendering these animals diabetic by the administration of phloridzin. According to BEURING, white rats become less immune to anthrax when confined exclusively to

vegetable food. The simultaneous infection with two germs, either of which would alone be harmless, may overcome a natural immunity. Rabbits succumb to symptomatic anthrax when inoculated simultaneously with the germ of this disease and living cultures of the bacillus prodigiosus. ALESSI has found that rabbits, guinea-pigs, and rats become highly susceptible to typhoid fever after having been compelled to breathe for some weeks the gases given off from decomposing excreta. Pigeons contract anthrax easily when the inoculations are made after prolonged deprivation of food, and sheep that have been repeatedly bled become more susceptible to the same disease. These and other illustrations that might be given show that natural immunity is a relative condition depending upon several influences. These facts suggest to us certain factors in the evolution of natural immunity. Suppose that the water-supply of a city is contaminated with typhoid bacilli. Not all of those who drink of this water will acquire the disease. In the first place, the quantity of the typhoid germ taken by the individual is a factor in the causation of the disease. Since the germs are not distributed uniformly in the water, and since the volume of water taken by each of two persons may be quite different, it is not strange that one acquires and the other escapes the disease. The one who gets the germ but escapes the disease acquires in all probability some degree of immunity to typhoid fever. At least we have the right to draw this inference from the results that we secure when we attempt to render animals immune to typhoid fever. In another individual drinking of the same water-supply escape from disease may be due not to the quantity of the germ ingested, but to the robust health and power of resistance of the individual. Again, in this case, it is altogether probable that lessened susceptibility results. It must be that in this way natural immunity is slowly acquired, and after many generations is transmitted from parent to offspring, until it happens that the latter is immune at birth. In other words, natural immunity to some diseases is an inherited acquired immunity. We cannot say that natural immunity

is universally evolved in this way. Indeed, there are many reasons for believing that some animals possess a physiologic immunity against certain diseases. The carnivorous animals probably owe their freedom from the intestinal forms of anthrax to the strong acid of the gastric juices. Wolves and other beasts of prey may feed upon sheep and cattle, sick or dead, with anthrax, and the millions of bacilli taken in this food are destroyed in the stomach of the consumer. That there are physiologic guards against infection, and that these differ in efficiency in different species and against different germs, there can no longer be any doubt. Indeed, these physiologic germicides and antitoxins are variable in strength among individuals of the same species and in the same individual at different times. These variations are determined in part by age and by the general health of the tissues which supply the germicides and antitoxins. While the adult white rat is naturally immune to anthrax, the young of the same animal are susceptible. The child is highly susceptible to diphtheria and scarlet fever, while the adult, though not wholly immune, has lost very much in susceptibility, and is likely to become infected only when much reduced in vitality or after prolonged and aggravated exposure. The only explanation of this immunity is that it is inherited from the parent cell, and comes as naturally as do the changes in form and voice at puberty, or the growth of the beard in early manhood. The evolution of this condition of immunity is due to the natural development of the functional activity of certain cells of the body. The cause of the difference in the effect of the anthrax-bacillus on the young rat and that of the same germ on the adult rat exists in the rat and not in the bacillus. A child and an adult are exposed to the Loeffler bacillus from the same source: the former becomes infected, the latter does not. The germ is the same, but in the development that converts the child into the adult the resistance with which the germ meets has been strengthened. The immunity that comes with adult life must be due to altered cell-activity.

The active agents that give natural immunity are germicidal and antitoxic, in some instances the former and in others the latter. The germ may be destroyed when introduced into the body of the immune animal, or it may for a while retain life and even multiply, but it is robbed of its virulence. The nature of these germicidal and antitoxic substances will be discussed later. It is enough to state here that natural immunity is essentially cellular, and that the formation of bactericides and antitoxins is dependent upon the cellular activity of certain tissues of the invaded host.

ARTIFICIAL OR ACQUIRED IMMUNITY.—Artificial or acquired immunity may be induced by several methods, some of which will now be discussed.

1. By an attack of an infectious disease ending in recovery.

Until the discovery of JENNER this was the only known cause of acquired immunity, and even now, so far as man is concerned, it is supposed to be the most potent cause. However, this form of immunity is only relative and not absolute. A man may have smallpox a second time, provided that several years have elapsed since the first attack, and provided that the second exposure brings him in contact with a highly virulent form of the infection, or the exposure continues through a long time or occurs when the health is reduced. The period of immunity given by an attack of some of the infectious diseases is so short that many have reasonably questioned its existence. It is generally believed to be true that the more grave and virulent is the disease, the greater and more persistent is the immunity. This indicates that there is a quantitative relation between cause and effect in the production of this form of immunity. It should be borne in mind that in inducing this immunity the substance of the germ is introduced into the body. This method found a practical application in inoculation-smallpox.

2. By vaccination with a modified and less virulent form of the germ, or by the introduction of at first a small quantity

of the germs and by subsequent successive inoculations with larger numbers.

Vaccination for smallpox, as discovered by JENNER and as now practised, most probably is an example of this method of inducing immunity. It has been claimed by some that vaccinia in the cow is a disease wholly distinct from smallpox, but the weight of evidence, supported by analogy from other diseases, renders it highly probable that the cause is the same in both instances, and that the specific virus is attenuated by its passage through the body of the cow.

TOUSSAINT rendered animals immune to anthrax by inoculating them with the blood of an animal dead from anthrax, heated to 55° for ten minutes. CHAUVEAU secured a like result by inoculations with diluted anthrax-blood, thus reducing to a small number the germs introduced, also by inoculating first with anthrax-blood heated to 50° for fifteen minutes, and later with blood heated to the same temperature for ten minutes. Later he employed pure cultures instead of anthrax-blood, which was objectionable on account of the difficulty experienced in maintaining every portion of the blood at the same temperature. The same investigator also found that cultures grown at 38° , under a pressure of eight atmospheres, became sufficiently attenuated to serve the purpose of a vaccine. PASTEUR's method of attenuating the anthrax-bacillus is as follows: The "first vaccine" consists of cultures that have been grown for twenty-four days at from 42° to 43° , and the "second vaccine" of cultures grown for twelve days at the same temperature. The anthrax-bacillus may be attenuated by exposure to germicidal solutions sufficiently diluted not to kill the germ, but strong enough to diminish its virulence, such as a one per cent. solution of carbolic acid. PASTEUR's method of vaccination against anthrax has now been in practical use for some thirteen years, and, according to CHAMBERLAND, the mortality has been reduced in France among sheep from 10 per cent. to 0.94 per cent., and among cattle from 5 per cent. to 0.34 per cent.

The Spanish physician FERRAN first vaccinated against

cholera. His experiments were made first on guinea-pigs and then on men, more than 30,000 of the latter being thus treated. He employed a virulent culture obtained from the stools of the sick. The first inoculation consisted of eight drops of such a culture and the second of 0.5 c.c. However, in many of the cases one c.c. was injected into each arm. Although FERRAN was bitterly assailed, especially by the French and Italian Commissioners sent to investigate his claims, subsequent researches have tended to the confirmation of his statements. The numerous epidemics of cholera in Europe during the past ten years have led many bacteriologists to study the methods of protection against cholera, and on the lower animals, at least, the claims of FERRAN have been substantiated. In 1892 FERRAN recommended that the people be vaccinated in mass against cholera by the addition of attenuated cultures to the drinking-water. In making this very questionable recommendation he assumes, as he has no right to do, that the attenuated cultures would not acquire virulence, and that man might be protected by way of the stomach as well as by subcutaneous inoculations. Indeed, the weakest point in all the work on vaccination against cholera rests upon the last of these assumptions. This point will be again referred to.

In 1888 GAMALEIA prepared cholera-vaccines consisting of cultures attenuated or sterilized by high temperature, and demonstrated their protective action on lower animals. ZÄSLEIN accomplished similar results by vaccinating with small quantities of virulent cultures. The cholera-vaccines of HAFKINE are obtained in two ways: (1) by growing the cultures in an atmosphere containing an excess of oxygen at 39° , and (2) by rubbing up agar-cultures with a 0.5 per cent. solution of carbolic acid. BRIEGER, KITASATO, and WASSERMANN ascertained that the cholera-bacillus, as well as other pathogenic microorganisms, are attenuated when grown in an infusion of the thymus glands of calves. The cholera-antitoxin of THEODORE is prepared by growing the cholera-germ for seven days in thymus-bouillon at 35° , then heating for fifteen minutes at 65° , and collecting the sediment that forms

and dissolving it in glycerin. KLEMPERER states that the cholera-germ may be attenuated by passing through the culture for twenty-four hours an electric current of twenty milliamperes. The blood of persons recovered from an attack of cholera has been found by FERRAN and others to have immunizing properties. These are the methods that have been employed in obtaining vaccines for cholera.

These cholera-vaccines have been introduced into the body : (1) By subcutaneous inoculation. The lower animals are speedily rendered immune by this method. In many of them the subcutaneous injection of a vaccine protects to such an extent that an ordinarily fatal dose of a culture of the germ of full virulence may be made sixteen hours after vaccination without ill effect. However, if a strong vaccine is employed, necrosis of tissue about the point of inoculation may result. This is avoided by using two vaccines, a weak and a strong one, with an interval of one day or more. (2) By injecting the vaccine into the peritoneal cavity. This method also gives a speedy and effective immunity. (3) By intravenous injection of the vaccine. This frequently causes death by the formation of emboli. (4) By administration of the vaccine through the mouth.

An interesting question arises in regard to these methods of vaccination. Do subcutaneous and intraperitoneal vaccination protect against infection by way of the mouth? If this question must be answered in the negative, these methods of vaccination can be of no practical benefit to man, because naturally man is infected only by way of the alimentary canal. What is the evidence on this point? Certain difficulties are encountered in the experimental study of this question. Most of the lower animals can be infected by the mouth only after being placed under certain artificial conditions. First we have the statistics of FERRAN. Of these, SHAKESPEARE, the American Commissioner who investigated the matter, makes the following statement: "From the Government statistics of cholera throughout the province of Valencia, it appears that among the villages invaded there were 62 attacks per thou-

sand of the population, and 31 deaths per thousand, which gives a mortality of 50 per cent. of those attacked. It appears from analysis of published official statistics of cholera in 22 towns where inoculation was performed, the inhabitants were divided as follows: 104,561 not inoculated, 30,491 inoculated. Of the latter there were 387 attacks of cholera, or 12 per thousand, and 104 deaths, or 3 per thousand; the mortality of those attacked being 25 per cent. Of the former there were 8406 attacks, or 77 per thousand, and 3512 deaths; being a mortality of those attacked of 43 per cent. It appears, therefore, that among the population of villages wherein anti-choleraic inoculations had been more or less extensively performed the liability of the inoculated to attacks of cholera was 6.06 times less than that of the non-inoculated, whilst the liability of the inoculated to death by cholera was 9.87 times less than that of the non-inoculated. These figures are based exclusively upon the data furnished by inoculations, the re-inoculations being left out of consideration because they are much less numerous, although from the records of the inoculations it would seem that the liability of attack, and especially of death, by cholera is many times less among them than among those inoculated a single time."

HAFKINE had, up to January, 1895, inoculated 500 persons in India (who were subsequently exposed or supposed to be). Of these, 21 were attacked and 19 died. At the same time, of 1735 non-inoculated persons exposed, 174 were attacked and 113 died. This gives a mortality among the vaccinated of 3.80 per cent., and among the unvaccinated of 6.51. With so great a difference in the number of the two classes, these figures are not of any special significance.

So far as we have experimental evidence from studies on the lower animals, we must conclude that in these at least subcutaneous and intraperitoneal vaccinations, while they give immunity against the virulent germ introduced in the same way, do not protect against intestinal infection. MERSCHNIKOFF has discovered that a true intestinal cholera may be established in suckling guinea-pigs and rabbits by adding

a few drops of a virulent culture to their milk, and that subcutaneous and intraperitoneal vaccinations do not protect against infections by way of the intestines. The Russian marmot, *spermophilus guttatus*, develops a violent intestinal cholera on receiving by mouth the cholera-germ, and SABOLOTZKY finds that in these animals also subcutaneous and intraperitoneal vaccinations fail to give immunity to the intestinal infection. From these facts some conclude that intestinal cholera and cholera-peritonitis are distinct diseases, although caused by the same germ. The former is the disease which afflicts man and the latter is an artificially induced disease, and that immunity to the latter does not protect from the former. METSCHNIKOFF has given to men by the mouth first a few drops of an attenuated, and later a like amount of a more virulent culture, and he states that there is no evidence of the production of immunity. He believes that the immunity or susceptibility of a given individual to cholera depends upon the other germs present in the intestines, some of which aid, while others prevent, the development of the disease.

Is the immunity so easily established against cholera-peritonitis specific or not? In other words, can it be induced by vaccination with other microorganisms than the cholera-bacillus, or with other chemie substances than the products of this bacillus? HUEPPE protects animals against cholera-peritonitis by previous injections of certain enzymes, such as papain. KLEIN secures the same results with sterilized cultures of the *b. prodigiosus*, *b. coli*, *b. typhi*, and *proteus vulgaris*. FRAENKEL and SOBERNHEIM deny that this immunity is in any way specific. Vaccination with the vibrio Metschnikovi protects against cholera (PALMIRSKI, WEIBEL).

PFEIFFER and ISSAEFF state that while it is true that animals immunized against the *b. pyocyaneus*, *b. coli*, *b. typhi*, and *proteus* are also immune to the cholera-vibrio, yet this immunity is not so great nor so lasting as that induced by vaccination with the cholera-germ itself. They, therefore, claim that the immunity is specific. SANARELLI finds that

the blood of an animal immunized to the typhoid bacillus will protect another animal against cholera-peritonitis, and METSCHNIKOFF immunized guinea-pigs against cholera with the blood-serum of men who had never had the disease. On the other hand, PREIFFER states that if a mixed culture of the cholera and the Nordhafen vibrios be injected into the peritoneal cavity of a guinea-pig already immunized against cholera, and the contents of the cavity be examined after a few hours, all the cholera-vibrios will be found dead and all the Nordhafen vibrios alive and in good health. On the other hand, if the animal had been previously immunized to the Nordhafen vibrio, these would all be killed and the cholera-germs would remain unhurt. This statement should be confirmed before it is generally accepted. PREIFFER proposes that this experiment be resorted to to decide the true character of a suspicious vibrio. SANARELLI finds that true cholera-germs from different localities show no constant vaccinal reciprocity.

PASTEUR attenuated the germs of chicken-cholera by exposing the cultures to the oxygen of the air for some days. TOUSSAINT accomplished a like result by passing the germ through the body of a rabbit, or by inoculating the fowl with the blood of a rabbit just dead from the effects of the germ, the latter animal being more susceptible than the former. KITR has ascertained that an immunizing substance is present not only in the blood and tissue-juices, but also in the eggs of vaccinated fowls. Not one of those methods has proved to be of practical value.

PASTEUR'S vaccine for swine-erysipelas, or ranget, is obtained by passing the germ through rabbits. This vaccine has been employed in France for more than seven years, and, according to CHAMBERLAND, the mortality has been reduced from twenty to one and one-half per cent.

For more than forty years the Boers of South Africa have practised inoculation against pleuro-pneumonia in cattle. Some of the fluid from the pleural cavity of an animal dead from the disease is inoculated in the tails of the healthy.

However, the death-rate from the protective inoculations has been high on account of the virulence of the germ, and this has led to a modification of the method. One of the herd is inoculated in the tail, as stated above, and as soon as a swelling appears at the point of inoculation matter is taken from this and is used in the protection of the others. The bacillus of pleuro-pneumonia has not been identified unless it be that of ARLOING, but, whatever it may be, the virus of this disease seems to become attenuated in passing through a series of animals.

As early as 1882 STERNBERG showed that the diplococcus of pneumonia could be so attenuated under the action of dilute germicidal substances that it might manifest a protective influence. This has been repeatedly confirmed, and vaccination against the septicemia induced in lower animals by this germ has been the subject of frequent study and will be again referred to in this chapter.

The above-mentioned examples illustrate some of the methods of inducing immunity with attenuated germs. By this method the germ that causes the disease is employed in attenuated form to induce immunity.

3. By one or more treatments with sterilized cultures of the germ or with the blood-serum of an animal possessed of either natural or acquired immunity to the special disease.

The production of immunity by the employment of chemical agents is a subject specially germane to this book, and some details must be given. We will first consider the immunization of animals against some poisons not of bacterial origin.

In 1887 SEWALL reported that he had immunized pigeons against the poison of the rattlesnake. That this author comprehended at that time the significance of the results which he obtained is demonstrated by the following quotation: "This work was undertaken with the hope that it might form a worthy contribution to the theory of prophylaxis. I have assumed an analogy between the venom of the poisonous serpent and the ptomain produced under the influence of bacterial organisms." Having ascertained the minimum fatal

dose, SEWALL administered at first less than this quantity, and by gradually increasing the size of the dose established an immunity which enabled the pigeons to bear without injury seven times the fatal quantity. "The prophylaxis gradually fails after the inoculations are discontinued; for an amount of the poison which may be injected at the end of a series of inoculations may be fatal if given a few months after the prophylactic treatment has ceased. It has been found, however, that immunity from the effects of ordinarily fatal doses of the venom is retained over a period of at least five months after the cessation of the course of preventive inoculation."

Recently (1895) FRASER has experimented with the venom of the cobra of India, *Naja tripudias*, the rattlesnake of America, *Crotalus horridus*, a large colubrine snake of Australia, probably a species of *Diemenia*, and the "rinkas" of Africa, *Sepedon haemachates*. The lethal dose of the dried cobra poison per kilogram of body-weight of animal was, for guinea-pigs, 0.00018 gram; for rabbits, 0.000245 gram; for white rats, 0.00025 gram; for cats, 0.005 gram; and for the grass snake, 0.03 gram. For rabbits the lethal dose per kilogram is, of the *Crotalus* venom, 0.004 gram; of the *Sepedon*, 0.0025 gram; and of the *Diemenian*, 0.0015 gram. However, all of these venoms were mixed with more or less foreign matter. These venoms exert a duplex action, one of which manifests itself in local irritation and the other induces functional disturbances based upon no recognizable lesions. The *Crotalus* venom induces the most marked local effect. When injected subcutaneously "the underlying muscles are reduced to an almost pulpy blood-stained substance, and post-mortem decomposition occurs soon after death."

By beginning with less than lethal doses and gradually increasing the amount, FRASER induced with the cobra-venom an immunity capable of withstanding fifty times the ordinarily fatal quantity. The immunity covers both factors in the duplex action. There is neither local irritation nor functional disturbance. Indeed, some of the animals took on flesh and seemed markedly benefited by the immunizing treatment.

“When an animal has acquired a resistant power over the minimum lethal dose of one venom, that animal is also able successfully to resist the lethal action of a dose above the minimum lethal dose of other venoms. To a rabbit immunized by cobra-venom, a dose above the minimum lethal of sepedon-venom has been administered; to rabbits immunized with crotalus-venom, doses above the minimum lethal of diamantina- and of cobra-venoms have been given; to animals immunized above the minimum lethal with the diamantina-venom, doses above the minimum lethal of crotalus- and sepedon-venom have been given; and in each case the animal has recovered and but few symptoms of injury were produced. At the same time, in other experiments, indications were obtained that animals immunized with a given venom are capable of resisting that venom more effectually than the toxic effects of other venoms.”

From three rabbits that had been immunized against thirty times the minimum lethal quantity of the cobra-venom, blood-serum was obtained, and with this animals were immunized against all the venoms. FRASER calls this blood-serum anti-venene, and an injection of 0.004 of a cubic centimeter per kilogram sufficed to protect rabbits against the minimum fatal dose, and 0.8 cubic centimeter or more injected thirty minutes after inoculation of the minimum fatal dose prevented death, notwithstanding symptoms of poisoning had already manifested themselves. “I have also administered 1.5 cubic centimeter per kilogram of cobra-antivenene thirty minutes after a dose one-twelfth larger than the minimum lethal doses of the venoms, respectively, of the sepedon haemachates, the crotalus horridus, and the diamantina serpent, and the rabbits experimented on have recovered. This success is all the more remarkable when the intensely destructive local effect of each, but especially of two of these venoms, is recollected.”

CALMETTE has recently made an interesting and valuable contribution to our knowledge of the action of venoms. He finds that the longer the snake is kept without food the more

deadly its poison becomes. Besides the points already mentioned, CALMETTE discusses the following:

1. Dilute solutions of hypochlorite of lime and of chlorid of gold destroy or neutralize venoms *in vitro*.

2. The toxicity of venoms is not destroyed by a temperature of 80°.

3. The blood of all snakes, of the salamander, and of the toad is poisonous.

4. The toxicity of the blood is destroyed by a temperature of 68°. Therefore, the toxicity of the blood and that of the venom is not due to the same constituent.

5. An animal may be immunized against the venom or against the blood, but immunity to the venom does not give immunity to the blood.

6. The mongoose (a species of ichneumon) is relatively to other animals of its size immune to the venom of serpents. Possibly some immunity is also possessed by the hog, but there was no opportunity to demonstrate this.

7. The blood-serum of the mongoose is slightly antitoxic to venom, and when both are injected simultaneously death is delayed. Five or more cubic centimeters of the blood-serum of dogs protected rabbits against the ordinarily fatal dose of venom.

8. An animal immunized against venom is not immune to tetanus; while antitetanic serum from the horse does retard or wholly prevent the action of venom.

9. Antivenomous serum retards the poisonous action of abrin and sometimes wholly prevents it. Antiabrin serum is also destructive to venom *in vitro*, but not protective *in vita*.

10. "Animals vaccinated against erysipelas or against rabies have a serum so active on venom that in certain cases it may even give immunity."

These facts show that the venom of animals immunized against a certain virus or poison may be immune to another virus or poison. They show also that the degree of resistance of an animal is not always correlative to the antitoxic power of its serum against the virus or poison to which it has been immunized."

“Can we conclude from this that the antitoxic serums have no real specificity, and are we right in hoping that some day an ideal serum will be discovered, giving immunity to the most formidable poisons?”

“This hypothesis, however seductive, seems hardly admissible, for we know of no serum capable of modifying different toxins equally. For instance, the antivenomous serum is much more active than any other serum or venom, and we have seen that the same is true of abrin, tetanus, and diphtheria.”

The fact that venom bears a relatively high temperature enabled CALMETTE to determine whether or not the antivenomous serum really neutralizes the venom *in vitro*. One milligram of venom mixed with 3 cubic centimetres of the serum, allowed to stand for ten minutes, and then injected into a rabbit, produced no ill effect. The same mixture after having been heated to 68° for ten minutes killed rabbits. This demonstrates that in this instance, at least, the antagonism between the venom and the antivenomous serum is physiological and not chemical. It should be noted that PRUSALIX and BERTRAND (1894) showed that viper-venom heated for fifteen minutes at 75° does not kill guinea-pigs. An exposure of five minutes at 80° gives like results. Furthermore the heated or attenuated venom confers immunity to animals.

In 1891, ENRLICH succeeded in establishing in animals a high degree of immunity against two of the most potent vegetable poisons known—ricin from the castor bean, and abrin from the jequirity bean. One gram of ricin is sufficient to kill one and one-half millions of guinea-pigs, and the potency of abrin is about one-half that of ricin. Immunity was easily established by feeding the animals upon small and gradually augmented doses. These poisons are apparently proteid, and in this respect, as well as in their great toxicity, resemble the venom of snakes and the toxins of the pathogenic microbes. Immunity established against one of these poisons does not hold good against the other. As between the two the immunity is specific. Later, ENRLICH ascertained that the

nursing young of an immune mother secured immunity with the milk.

The preceding statements concerning immunity to non-bacterial poisons have been given on account of the light which they throw upon the production of immunity to the infectious diseases established by the employment of sterilized cultures and the blood-serum of animals already rendered immune.

When TOUSSAINT, in 1880, rendered animals immune to anthrax by treating them with defibrinated blood of animals dead with the disease, heated for ten minutes at 55° , he employed a sterilized culture, and this probably was the first time that such cultures were used for this purpose. In 1886, SALMON and SMITH immunized pigeons to the bacillus of hog-cholera with sterilized cultures, these animals having naturally only slight susceptibility to the living bacillus. FRAENKEL and SIMMONDS, and BEUMER and PEIPER, in their studies of the etiological importance of Eberth's bacillus, observed that when an animal recovered from the effects of sterilized or unsterilized cultures, and was again used, it bore the second time a much larger quantity of these cultures than that ordinarily required to cause death. The observation was so patent in the experiments of BEUMER and PEIPER that they began with small doses, and gradually increasing them secured a high degree of immunity. In 1887, ROUX and CHAMBERLAND immunized animals against malignant œdema and symptomatic anthrax with sterilized cultures, and CHANTÉSE and WIDAL did the same with the Eberth bacillus.

In the same year FOA and BONOME announced to the Academy of Medicine at Turin some very important observations.

Not only had they rendered animals immune to the proteus vulgaris, diplococcus of pneumonia, and the bacillus of chicken-cholera by treating them with sterilized cultures of these germs, but they had discovered that the blood taken from the heart or an infusion of the tissues of a rabbit dead from proteus infection injected intravenously into another rabbit made this animal immune to virulent culture of the proteus. More-

over, they ascertained that the blood and tissue-infusion, with which they induced immunity, formed good culture-media on which the proteus grew abundantly and remained possessed of full virulence. This protective blood had no germicidal properties.

In 1890 BEHRING began his work on immunity and cure, a very brief *résumé* of which can be given here. Animals were rendered immune to diphtheria and tetanus by the subcutaneous injection of the specific toxins in gradually augmented doses. In this manner the resistance of the animal could be increased a thousandfold, and the blood-serum of such an immunized animal could be employed not only to give immunity to men and animals, but to effect cures.

The effect of large quantities of virulent cultures of the diphtheria-bacillus upon animals protected in different degrees with the serum is thus described: If one injects into a guinea-pig, which has just been infected with ten times the fatal dose of a diphtheria-culture, an amount of the normal curative serum in the proportion to the body-weight of the animal of 1 : 5000, death does not result, but the animal sickens. At first the sickness is similar to that in a control-animal. There is a local œdema that becomes harder and more extensive day by day, and is filled with a firm, fibrinous exudate. Generally about the eighth day, sometimes only after some weeks, a line of demarcation forms about the tumor, which is often as large as a child's hand. The separation continues until the skin pulls off, leaving a permanently bare spot which covers over with scar-tissue. With the same large quantity of the virulent culture, but with an increase in the amount of the serum to the proportion of 1 : 2000, there is a slight infiltration which is soon absorbed, and nothing abnormal can be observed after ten days. With the serum increased to 1 : 500, the animal remains wholly free from any evidence of the disease.

After the animal has been thoroughly immunized, portions of blood are drawn from time to time under aseptic precautions. The blood is received in sterilized flasks, the bottoms of which are covered with chloroform. These flasks are

allowed to stand in an ice-chest until the serum separates. The serum is then poured off, and 0.5 per cent. of carbolic acid (or a proportional amount of some other preservative) is added. Serum thus obtained should preserve its antitoxic properties quite indefinitely.

The protective value of the serum might be estimated quantitatively according to either of the following standards:

1. Its immunizing power against infection.
2. Its curative power against infection.
3. Its immunizing power against intoxication.
4. Its curative power against intoxication.

1. In order to determine its immunizing power against infection, a very virulent culture of the diphtheria-bacillus is prepared, and the smallest fatal quantity of this per kilogram of body-weight of guinea-pig is determined by experiments on numerous animals. This being ascertained the work is facilitated, and time saved by employing ten times the smallest fatal dose as the unit in estimating the strength of the serum, because if the smallest dose was used death would result only after many days, and some animals would escape death from the smallest ordinarily fatal dose. By taking ten times the smallest fatal dose death is certain in the control-animals, and whether or not the treated ones will succumb is known after a delay of a few days at most. The difficulties met with in making this determination are the following: 1. The diphtheria-culture should be of great virulence, and such cultures cannot always be obtained at once. 2. Having obtained a culture of the desired virulence, it does not continue of constant strength, but is likely to change from day to day, or on account of a variation of a few degrees in the thermostat in which it is kept it may quickly become worthless. This determination, therefore, is not made in the preparation of the serum.

2. To determine the curative value of the serum against infection would be a still more complicated task and the results less certain. The virulence of the culture, its age, the temperature at which it had been kept, the lapse of time

between the infection and the beginning of the treatment, are factors which it would not always be possible to measure, and which would affect the results.

3. The determination of the immunizing power of the serum against intoxication is easily made and gives uniform results. This is the quantitative measure which is now employed in the preparation of the serum. A culture of marked virulence is prepared and sterilized. This forms a diphtheria-toxin which will retain its properties unchanged indefinitely. It can be kept for months, and employed in determining the strength of any number of samples of serum. The smallest fatal dose of this is determined, and, for reasons already given, ten times this amount is used as the unit for determining the strength of the serum.

4. BEHRING and his associates made frequent tests of the serum as a curative agent in animals to which a more than fatal dose of the toxin had been administered, and in which the symptoms had already developed. Even when death seemed imminent a dose equivalent to six times the amount necessary to immunize against the same quantity of the toxin saved some of the animals.

BEHRING records the following noteworthy observations made while immunizing animals: In immunizing sheep to tetanus he found that it sometimes happened that the blood no longer contained any antitoxin, and yet the animals possessed a higher degree of immunity than they did when the blood contained the greatest quantity of antitoxin. The same thing has been observed by SCHÜTZ in horses rendered immune to tetanus. Indeed, a horse in whose blood the antitoxin had decreased a hundredfold in the course of a year was at this time so thoroughly immune to the disease that no culture could be found sufficiently virulent to cause the slightest reaction. This shows that immunity is not dependent upon the antitoxic properties of the blood. The living tissues had in these animals acquired an immunity similar to that possessed by animals naturally immune. Immunity may, therefore, be due to insusceptibility of the tissues. The first

may be called hæmatogenous, and the second histogenic. The former is not transmissible from father to offspring. Whether acquired histogenic immunity can be thus transmitted or not has not been experimentally determined. The probabilities are that these are really not different forms, but different degrees of immunity. On the other hand, BEHRING observed that during the process of immunization some animals became exceedingly hypersusceptible, and this happened at a time when the blood was antitoxic to a high degree.

"This can go so far that a horse may have in one c.c. of its blood enough antitoxin to protect an untreated horse from a quantity of the poison, a fraction of which would kill the animal supplying the antitoxin."

Experimental immunity has been established in one or more species of the lower animals by the employment of sterilized cultures of the specific bacteria in anthrax, symptomatic anthrax, cholera, chicken-cholera, hog-cholera, diphtheria, erysipelas of the hog, influenza, pneumonia, pleuro-pneumonia, swine-plague, streptococcus infection, tetanus, and typhoid fever. BABES states that he has rendered guinea-pigs immune to glanders by the employment of sterilized cultures. However, mallein has not yet proved of signal value either as a protective or curative agent, and its sole use is for diagnostic purposes. HERICOURT and RICHET claim to have immunized rabbits to tuberculosis by the intravenous, and also by the subcutaneous injection of sterilized cultures, and DE SCHWEINITZ states that he has successfully vaccinated guinea-pigs against this disease by the employment of attenuated cultures. On account of the well-known fact that guinea-pigs are very susceptible to inoculation-tuberculosis, full credence should not be given this statement until it has further confirmation. This caution is applicable to the above-mentioned claim of HERICOURT and RICHET also, inasmuch as only half of their control-rabbits failed to develop tuberculosis. This shows that the bacillus employed in these inoculations could not have been very virulent.

BRUSCHETTINI grows the influenza-bacillus in blood, filters these cultures, and employs the filtrate in rendering animals immune. From the immunized animals he claims to have obtained a serum of great strength not only as an immunizing, but as a curative agent.

In all of the preceding methods of inducing immunity the germ or the poison against which the immunity is to be induced is introduced into the body. In the production of immunity (1) by one attack of the disease, (2) by vaccination with attenuated cultures or with small quantities of virulent cultures, and (3) by one or more treatments with sterilized cultures—in all of them—the germ itself living or dead is the immunizing agent. The difference between the living and dead germ is one of degree rather than of kind. The poison is the same. In the living germ the poison is capable of growth and increase in quantity. In the dead germ the amount of the poison is not increased after it is injected into the animal. Even when the blood-serum, tissue-juice, or milk of one immune animal is employed to render a second immune, the germ-poison is the immunizing agent. The second animal has no immunity of its own. That which it has is borrowed from the first, and will soon be lost. The second is for the time physiologically a part of the first. There is this, however, which may give the borrower actual possession of the immunity: When the second animal is inoculated with the germ in order to prove its immunity, and when on account of its borrowed power it resists the infection, then it may acquire an immunity of its own.

From the above-mentioned facts we conclude that certain poisons, the venom of snakes, the blood of snakes, toads, and salamanders, certain vegetable poisons, as abrin and ricin, and the bacterial toxins awaken, and when repeatedly introduced in non-lethal doses develop, in the animal body an antagonistic action which tends to protect the body against the effect of these poisons.

The difference between immunity and tolerance we believe to be this: In the former the cells of certain organs of the

body become aggressive; a special function is developed; the poison introduced is destroyed. In tolerance there is no aggressive action on the part of any organ; there is no development of special function; the poison introduced is not destroyed, it only fails to kill.

ANTITOXINS.—We know very little concerning these substances. They are believed to be proteid bodies, but this belief is not founded upon any positive knowledge. So far we can only recognize their presence by their effects. BEHRING finds that the diphtheria-antitoxin is not altered by peptic digestion. If this be true, it cannot be serum-albumin or globulin, but must be a nuclein, since this is the only proteid-like body in the blood that is not altered by peptic digestion. BEHRING believes that the action of antitoxin on toxin is a chemie one, and he compares it to that of an acid on an alkali, or to that of soluble sulphates on phenol in the body. BUCHNER combats this idea on experimental grounds. He finds that a mixture of tetanus-toxin and antitoxin, which has no effect on mice, kills guinea-pigs, and he argues from this that if the antagonism was a chemie one a mixture of given quantities of toxin and antitoxin found to be harmless to the mouse should also be harmless to other animals. This reasoning seems to us to be good and conclusive. There is no positive proof that toxins and antitoxins neutralize, one with the other, *in vitro*. Again, as pointed out by ROUX, a mixture of the diphtheria-germ and antitoxin injected into an animal previously treated with *B. prodigiosus*, streptococci, etc., is fatal, although a similar mixture injected into a normal non-treated animal is without effect. Because a mixture of these substances that has been kept in a test-tube for so many minutes fails to produce any effect when injected into an animal, is no proof that neutralization has taken place *in vitro* or is chemie; it may have taken place *in vita*, and be physiologic. In fact, BUCHNER's and ROUX's experiments furnish strong evidence in support of the view that the antagonism is physiologic. This certainly is true of snake-venom

and the antitoxin of antivenomous blood-serum, as demonstrated by CALMETTE.

The theory has been advanced that antitoxin is the toxin modified by the fluids and tissues of the body. This may possibly be true. The evidence against it is not altogether conclusive. It may be stated as follows: 1. The taking of large quantities of blood, one-third or more of the contents of the body, from immunized animals, at intervals of time sufficiently long to allow the animal to recover to such an extent that its health is not seriously impaired, does not diminish the immunity of this animal as markedly as would be expected if the antitoxin was a modified toxin, and the quantity of the former could not be greater than the quantity of the latter employed in immunizing the animal. 2. If an immunized animal be bled to death and its vascular system be washed with physiologic salt-solution until all the blood that can possibly be washed out is removed, yet infusions of certain tissues of this animal contain an antitoxin. To the first of these it may be answered that the blood may contain absolutely no antitoxin, as has already been stated, and yet the animal possesses the most marked immunity. To the second it might be answered that the tissues may retain some of the modified toxin in the form of antitoxin after the most thorough washing of the vascular system.

CENTANNI has endeavored to determine experimentally what organ or tissue of the body is affected in the production of immunity against rabies. His work consisted in endeavoring to induce immunity in a second series of rabbits by treating them subcutaneously with the blood-serum and with emulsions made from the various organs of the members of a primary series already rendered partially or wholly immune by the ordinary method. In doing this he has used amounts of each tissue proportional to the weight of the animal. Thus, the central nervous system of an average rabbit is equal in weight to one three-hundredth part of the total body-weight of the animal, and the total blood of the same animal furnished an amount of serum equal in weight to one one-

hundred-and-fiftieth part of its body-weight. Therefore he injected into a rabbit weighing 1800 grams 6 grams of nervous tissue made into an emulsion, and into another of the same weight 12 grams of blood-serum taken from rabbits already made immune, and then tested the immunity of the members of this second series. Emulsions of other tissues were employed in proportionately the same doses. By this procedure he found, as he thinks, that in the production of immunity against rabies the immunizing substance is stored up in the central nervous system. Moreover, he found that the immunizing substance remains in the nervous tissue long after it has disappeared from the blood and other organs. Thus it would seem that PASTEUR hit upon the right thing exactly when he selected emulsions of the spinal cord as the proper material with which he could best induce immunity against hydrophobia. From these experiments, which need to be confirmed, CERRAXNI draws the general conclusion that in the production of immunity against any disease the immunizing substance is stored up in greatest quantity and most permanently in that organ or tissue most seriously affected by the disease. It is hardly necessary to add that a principle so general and so important as this cannot be safely founded upon a single series of experiments confined to one disease.

SMIRNOW has written quite at length to show that toxins may be converted into antitoxins by the long-continued action of an electric current. We fail to find in his recorded experiments any justification of his claim. He makes quite a point of the fact that during the continuance of the electrolysis the bouillon becomes more deeply colored at one pole and nearly decolorized at the other. Now, the merest tyro in physiologic chemistry knows that acids deepen the color of beef-tea, urine, or any other fluid the coloring-matter of which is derived from hæmoglobin. So much for his chemistry. His physiology is worse. When he administers this artificial antitoxin in too large doses he kills his animals; and of what do they die—of diphtheria? He has only modified and reduced

the virulence of his diphtheria-toxin by the acid generated by the electrolysis of the inorganic salts in the bouillon. Toxins may be convertible into antitoxins, and electricity may be the agent capable of inducing this, but SMIRNOW has proved neither one nor the other.

It is generally conceded that the germicidal and antitoxic properties of the blood of an immunized animal are not due to one and the same constituent. The blood-serum of a guinea-pig that has been immunized to the cholera-germ has both germicidal and antitoxic properties. If it be heated to 55° it is no longer germicidal, but is still antitoxic. If a second animal be immunized with this heated blood, the blood-serum of this animal is again both germicidal and antitoxic. From these facts it is inferred that the germicidal and antitoxic properties are due to two distinct constituents of the serum. This inference, however, does not necessarily follow. Both of these properties may be possessed by one and the same substance and the heat may destroy one property without destroying the other. The cholera-germ is possessed of life and is a poison. A moderately high temperature will destroy life, but the substance of the germ retains its poisonous properties.

PHAGOCYTES.—The theories of immunity can only be referred to here. The phagocytic theory was first suggested by STERNBERG in 1881, but METSCHNIKOFF, ignorant of STERNBERG's suggestion, in 1884 stated the same theory supported by experimental evidence, and since that time he has so masterly advanced and defended this theory that it is now generally known as METSCHNIKOFF's theory. In brief, this theory makes the polynuclear white blood corpuscles the chief defenders of the body against bacterial invasion. It had long been known that these amœba-like bodies take up small particles of carbon and other dust-particles that get into the tissues and blood, and METSCHNIKOFF, in the study of the daphnia, ascertained that these small animals are frequently infected with a torula-like organism that enters by way of

the alimentary canal. As soon as the invaders pierce the intestinal walls the polynuclear cells gather about and engulf them. If all of the parasites are taken up by the corpuscles, the daphnia escapes the disease. If the invaders are too numerous or the defending corpuscles too few, the host sickens and dies. These observations suggested to METSCHNIKOFF that possibly the higher animals might be supplied with like protective agents against bacterial infection. Investigation showed this to be a fact. If anthrax-bacilli be injected under the skin of a pigeon and the tissue about the point of inoculation be examined at different intervals of time, the following phenomena will be observed: After a few hours at most it will be found that a large number of polynuclear corpuscles have collected at this place, and many of these corpuscles, it will be observed, contain bacilli. The longer the time after the inoculation the more bacilli will be found in the corpuscles, until finally all are taken up in this way. If some days have elapsed, this material may be injected into a mouse or other animal highly susceptible to anthrax, and the fact that this animal suffers no inconvenience shows that the bacilli are dead. Some experiments by WEYL nicely illustrate these facts. Silk threads covered with the most virulent anthrax-spores were introduced beneath the skin of chickens and pigeons and left for from one to fifteen days. They were then removed and each thread cut into three pieces. One was placed in bouillon, the second on agar, and the third under the skin of a mouse. The spores on the threads left in the pigeon for six days or longer failed to grow in bouillon or on agar, and also failed to kill mice. The researches of TRAPEZNIKOFF carried on under the direction of METSCHNIKOFF show that when spores are introduced in this way under the skin of an immune animal the spores first develop into bacilli and the latter are taken up by the phagocytes. The theory of METSCHNIKOFF teaches that the polynuclear white blood-corpuscles feed upon and digest the invading bacilli. It is only fair to state that the author of this theory has never claimed that this method of defence is universal, or that the

animal body might not be possessed of other means of protection against bacterial invasion. He says: "Biologic problems are so complex that when a rule concerning them has been established one must expect more or less numerous exceptions. What is more common than the shell in mollusks or the teeth in vertebrates as means of defence, and yet how many exceptions. Some mollusks are bare or have shells as thin as paper and depend for safety upon their ability to swim rapidly. Others escape their enemies by the secretion of black ink. There are vertebrates that defend themselves with beaks or claws instead of teeth." We cannot go into all the evidence for or against the phagocytic theory. In our opinion there can be no question concerning the correctness of the observations upon which this theory is based. The polymuclear corpuscles certainly are the most important defenders of the body against the pathogenic germs. Whether they take up the living germs or first destroy them by means of a germicidal secretion and then absorb them are questions which, in our opinion, remain without positive solution. Even if it be true that the phagocytes take up and digest the living germs, this digestion must be accomplished by some chemie constituent of the phagocyte. The facts that nuclein is a prominent and abundant constituent of the phagocyte, that this nuclein is a powerful germicide, and that the germicidal properties of blood-serum are due to nucleins which come from the polymuclear corpuscles, lead us to formulate the following statements, which, in our opinion, most nearly express the facts as interpreted by the knowledge of the present time:

1. The phagocytes, both the wandering polymuclear corpuscles and the fixed endothelial cells, are the active defenders of the body against bacterial invasion.
2. The phagocytes destroy bacteria by virtue of the nuclein which they contain.
3. The germicidal properties of the humors of the body are due to nucleins formed by the phagocytes.

The relation of nuclein to immunity has been demonstrated

recently by VAUGHAN, who has rendered rabbits immune to the diplococcus of pneumonia by previous treatments with nucleinic acid obtained from yeast. The injections of nuclein increase the number of polynuclear cells and thus strengthen the resistance of the body. This method of inducing immunity is quite different from those that have been discussed in the preceding pages. In those the resistance of the body is strengthened by exercise; in this, by feeding.

SERUM-THERAPY. The preparation of curative blood-serum has already been discussed. ROUX made the preparation of antidiphtheric serum more easy and practical when he substituted the horse for the goat and sheep, previously used by BEHRING, as the source. The toxin with which the horse is to be immunized is prepared by growing the diphtheria-bacillus for thirty days in a flask so constructed that a current of air can be constantly drawn over the surface of the bouillon. These flasks are kept in an incubator. At the expiration of the time mentioned the fluid contents of the flask are decanted from the deposit, which consists principally of dead, disintegrating bacilli, 0.5 per cent. of carbolic acid is added, and the fluid thus obtained should be so toxic that 0.1 c.c. of it will kill a guinea-pig of 500 grams weight within forty-eight hours. Healthy horses are selected, and beginning with injections of from $\frac{1}{2}$ to 2 c.c. of the toxin-solution, the dose is gradually increased and given at intervals of from five to eight days, until one-fourth of a litre is injected without effect. (The rapidity with which the dose is increased by different experimenters is very variable, and a more speedy result may be obtained by treating the horse with living cultures, although by this method the danger of killing the horse is increased.) From time to time small quantities of blood are taken from the horse, and the immunizing power of the serum tested on guinea-pigs by the method already given. This curative serum has now been used in the treatment of many thousands of children, and the following facts seem to be demonstrated: 1. The treatment is practically free from ill-

effects. 2. The mortality has only been from one-half to one-fourth what it was during the year preceding the introduction of this agent. There are those who claim that diphtheria has suddenly decreased in virulence, and that by the bacteriologic method of diagnosis many cases, which formerly would not have been called diphtheria, have been included in the statistics, and that these are the causes of the apparently good results. However, the value of this method now rests upon evidence that seems to us to be conclusive.

In studying the results that have been obtained by the blood-serum treatment of tetanus, it is well to bear in mind, as has been suggested by ROTHIER, that there is a great difference in the rate of mortality in this disease under any treatment or without treatment, the longer the period between the injury and the first appearance of tetanic symptoms, or, in other words, the longer the period of incubation, the less fatal is the disease. When the period of incubation is less than ten days the mortality is about 96 per cent. With this period increased to more than twenty days, 50 per cent. may recover (RICHTER, ROSE). In the acute form the muscles of the whole body (with the exception of the arms) become tetanized in the highest degree within from a few hours to two days, and death results within from five to eight days. However, some of these cases become chronic, and death results from prostration or from failure of the organs of circulation or respiration. In tetanus mitis the symptoms develop slowly. There is a prodromal stage of several days, during which the patient complains of pain in the limbs and difficulty of locomotion. The tetanus of the muscles is slight, and recovery is not infrequent. In the localized trismus of Richter and the head-tetanus of Rose most of the cases recover. In general, however, the prognosis is bad. Of the 717 cases collected by RICHTER, 631 (88 per cent.) died, and 40 (12 per cent.) recovered. Of the 40 recoveries 13 consisted of localized tetanus.

Most of the cases of tetanus so far treated with serum that have recovered belonged to the milder form of the disease.

CHAPTER IX.

METHODS OF EXTRACTING PTOMAÏNS.

FROM what has been given in the preceding pages, one may gather some idea of the peculiar difficulties with which the chemist has to contend in his endeavors to isolate the basic products of putrefaction. He has to deal with very complex substances, of the nature and reactions of many of which he must be ignorant. Besides, the substances which he seeks are often most prone to undergo decomposition, and in this way escape detection. Many ptomaïns are volatile or decomposable at any temperature near that of boiling water. In these cases, solutions cannot be evaporated in the ordinary way and the poison separated from the residue. Indeed, the investigator has frequently been disappointed when on the evaporation of a solution, which he has demonstrated to be poisonous, he finds that the residue is wholly inert. Again, he may destroy the ptomaïn by the action of reagents which he uses. So simple a procedure as the removal of a metallic base from a solution containing a ptomaïn, by precipitation with hydrogen sulphide gas, has been known to destroy wholly the ptomaïn. Probably the most perplexing difficulty in the isolation of these putrefactive alkaloids lies in the great number, complexity, and diversity of the other substances present in the decomposing mass. The same ptomaïn may be present in equal quantities in two samples of milk, and yet it may be easily obtained from the one, while from the other only minute traces can be secured. The difference is due to the fact that the other constituents of the milk in the two samples are at different stages of the putrefactive process, and, consequently, differ greatly in their reactions and in their effects upon the agents employed to isolate the poison. All chemists will appreciate these difficulties.

One of the first things for the chemist who undertakes to do this work is to ascertain whether or not his reagents are pure. We have found a number of samples of German ether, which was imported on account of its supposed purity, to yield on spontaneous evaporation a residue which gave several of the alkaloidal reactions, and a few drops of which, injected under the skin of a frog, caused paralysis and death within a few minutes. We would advise that 500 c.c. of the ether to be used should be allowed to evaporate spontaneously, and its residue, if there be one, be examined both chemically and physiologically. The basic substance which exists in some samples of sulphuric ether is pyridin.

GUARESCHI and MOSSO found commercial alcohol almost invariably to contain small quantities of an alkaloidal substance, the odor of which is similar to that of nicotin and pyridin. Its solutions are precipitated by gold chlorid, phosphowolframic acid, phosphomolybdic acid, potassium iodid, and Mayer's reagent, but not by platinum chlorid or tannic acid. It does not reduce, or reduces feebly, ferric salts. From one sample of alcohol they obtained a base which, in addition to the above reactions, did give a precipitate with platinum chlorid. Alcohol may be freed from these substances by distillation over tartaric acid.

In amyllic alcohol, HARTINGER has found as much as 0.5 per cent. of pyridin. It may be purified in the same manner as recommended for ethylic alcohol.

Chloroform, when found to leave any residue on evaporation, should be washed first with distilled water, then with distilled water rendered alkaline with potassium carbonate, then dried over calcium chlorid and distilled.

Petroleum-ether sometimes contains a base which has an odor similar to trimethylamin or pyridin, and which gives a precipitate with platinum chlorid, forming in octahedra.

Benzol may contain a similar substance.

The following methods have been used for the purpose of extracting the putrefactive alkaloids:

THE STAS-OTTO METHOD.—This method depends upon the following facts: (1) The salts of the alkaloids are soluble in water and alcohol, and generally insoluble in ether, and (2) the free alkaloids are soluble in ether, and are removed from alkaline fluids by agitation with ether. These principles are capable of great variety in their application. The usual directions are as follows: Treat the mass under examination with about twice its weight of pure 90 per cent. alcohol, and from ten to thirty grains of tartaric or oxalic acid; digest the whole for some time at about 70° , and filter. Evaporate the filtrate at a temperature not exceeding 35° either in a strong current of air or in vacuo over sulphuric acid. Take up the residue with absolute alcohol, filter, and again evaporate at a low temperature. Dissolve this residue in water, render alkaline with sodium bicarbonate, and agitate with ether. After separation remove the ether with a pipette, or by means of a separator, and allow it to evaporate spontaneously. The residue may be further purified by redissolving in water, and again extracting with ether.

The following modifications of this method are employed: Instead of tartaric or oxalic acid, acetic acid is frequently used.

When the fluid suspected of containing a ptomain is already acid from the development of lactic or other organic acid, the addition of an acid is often dispensed with.

Ether-extracts are made from both acid and alkaline solutions.

Chloroform, amyllic alcohol, and benzin are used as solvents after extraction with ether.

The modification of this method, as carried out by SELMI and MARINO-ZUCO, is given in detail as follows:

The material is divided as minutely as possible, placed in a large flask, and treated with twice its volume of 90 per cent. alcohol, and acidulated with tartaric acid in the proportion of 0.5 gram to 100 c.c. of the mixture, taking care from time to time that the reaction is permanently acid. The flask, which is connected with a reflux condenser, is now placed on

the water-bath and kept at the constant temperature of 70° for twenty-four hours. While yet warm the liquid is transferred to a special apparatus for filtration by the aid of atmospheric pressure. The liquid is poured upon a wet cloth, supported upon a perforated porcelain funnel, which is connected below with a receiver exhausted by a water-pump or aspirator. In this way rapid filtration is secured, and by repeated washing the extraction is made thorough. The acid alcoholic liquid is now transferred to a special distillation-apparatus.

A large tubulated retort of ten litres capacity is connected by means of a cork to a large tubulated receiver. The tubulure of the retort is provided with a small perforated cork, which carries a glass tube finely drawn out and extending to the bottom of the retort. The tubulure of the receiver is connected with Leibig's bulbs containing dilute sulphuric acid (1 to 10), and the bulbs in turn are connected with a water-pump or aspirator.

In order to prevent the passage of air through the corks, they are covered with animal membrane which has been freed from fat. By means of the aspirator a fine current of air is drawn through the liquid and suffices to keep it constantly agitated. The retort is kept on the water-bath at a temperature of from 28° to 30° . The receiver is kept cold by a current of water. In this manner the distillation of the alcohol goes on rapidly and conveniently. Moreover, decomposition is so far prevented that volatile bases are never found in the bulbs.

The aqueous residue, after the removal of the alcohol by distillation, is filtered and extracted with ether as long as anything is dissolved. It is then mixed with powdered glass and evaporated to dryness in vacuo. This residue is repeatedly extracted with absolute alcohol. The alcohol is distilled again in the apparatus already described. The residue is taken up with distilled water and filtered. It is then made alkaline with sodium bicarbonate and repeatedly extracted with ether, benzin, and chloroform.

In order to obtain the base from the solvent, the greater part may be evaporated on the water-bath and the remainder allowed to evaporate spontaneously, or the remainder may be treated with dilute hydrochloric acid and the evaporation continued on the water-bath or in vacuo.

DRAGENDORFF'S METHOD.—The finely divided substance is digested for some hours with water acidulated with sulphuric acid at from 40° to 50° . This is repeated two or three times, and the united filtered extracts are evaporated to a syrup. This is treated with four volumes of alcohol and digested for twenty-four hours at 30° . After cooling the alcoholic extract is filtered, the residue washed with 70 per cent. alcohol, and the united filtrates freed from alcohol by distillation. The aqueous residue, diluted if desirable, is filtered and submitted to the following extractions:

1. The acid liquid is shaken with freshly rectified petroleum-ether as long as this reagent leaves any residue on evaporation.

2. The acid fluid is now extracted with benzin.

3. The next solvent used is chloroform.

4. The liquid is now again extracted with petroleum-ether in order to remove traces of benzin and chloroform.

5. The liquid is now made alkaline with ammonia and successively extracted with petroleum-ether, benzin, chloroform, and amyl alcohol.

6. The remainder of the ammoniacal liquid is mixed with powdered glass, evaporated to dryness, the residue pulverized, and extracted with chloroform.

The residue obtained with each of the above solvents should be examined for ptomains.

BRIEGER'S METHOD.—The substance under examination is divided as finely as possible, and then heated with water slightly acidified with hydrochloric acid. During the heating care must be taken that the feebly acid reaction is maintained. The heating should continue for only a few minutes. The

liquid is then filtered and concentrated, at first on a plate and then on the water-bath, to a syrup. If one has material which is highly odorous, as is the case frequently both with aqueous and alcoholic extracts of putrid material, BRIEGER recommends that a piece of apparatus devised by BOCKLISCH be used. The fluid to be evaporated is placed in a globular flask, the rubber stopper of which carries two small glass tubes. One of these, B, extends to the bottom of the flask, while A terminates just above the surface of the liquid. The tube, A, is connected with a water-pump or aspirator, which draws the vapor through the tube. In order to prevent the return of condensed fluids, the end of A in the flask is curved upon



itself. The tube B is finely drawn out and through it a current of air is constantly moving. This prevents the formation of a deposit or a pellicle in the fluid. By regulating the amount of air coming through this tube, more or less of a vacuum will be formed in the flask. After evaporation to a syrup, an extraction is made with 96 per cent. alcohol, and the filtered extract is treated with a warm alcoholic solution of lead acetate. The lead-precipitate is removed by filtration,

the filtrate evaporated to a syrup and again extracted with 96 per cent. alcohol. The alcohol is driven off; the residue taken up with water; traces of lead removed with hydrogen sulphid; and the filtrate, acidified with hydrochloric acid, evaporated to a syrup. This syrup is extracted with alcohol, and the filtrate precipitated with an alcoholic solution of mercuric chlorid. The mercury-precipitate is boiled with water, and on account of differences in solubility of the double compounds with mercury one ptomain may be separated from others at this stage of the process. (If thought best, the lead-precipitate may be freed from lead and carried through the following steps of the process. BRIEGER has found small amounts of ptomains in the lead-precipitate only in his work with poisonous mussels.)

The mercury-filtrate is freed from mercury, evaporated, and the excess of hydrochloric acid carefully neutralized with soda (the reaction is kept feebly acid); then it is again taken up with alcohol in order to free it from inorganic salts. The alcohol is evaporated, the residue taken up with water, the remaining traces of hydrochloric acid neutralized with soda; the whole acidified with nitric acid, and treated with phosphomolybdic acid. The phosphomolybdate double compound is separated by filtration and decomposed by neutral acetate of lead. This is hastened by heating on the water-bath. The lead is removed by hydrogen sulphid, the filtrate is evaporated to a syrup and taken up with alcohol, from which many ptomains are deposited as chlorids, or double salts may be formed in the alcoholic solution. BRIEGER states that the chlorids as deposited from the alcoholic solution are seldom pure, and he advises for their purification, precipitation with gold chlorid platinum chlorid, or picric acid, and, on account of differences in solubility of these double salts, the process of purification is rendered more easy. The chlorid of the base is obtained by removing the metallic base with hydrogen sulphid, while the picrate is taken up with water, acidified with hydrochloric acid, and repeatedly extracted with ether, in order to remove the picric acid.

THE METHODS OF GAUTIER AND ETARD.—The putrid matters, liquid and solid, are distilled at a low temperature in *vacuo*. The distillate (A) contains a considerable quantity of ammonium carbonate, some phenol, skatol, trimethylamin, and the volatile fatty acids. The residue after distillation is treated in succession by ether and by alcohol.

The extraction with ether (B) separates the ptomaines and some fatty acids. The alcoholic extract (C) removes the remainder of the fatty acids, as well as the acid and neutral nitrogenized bodies, almost all of which are crystallizable. The insoluble residue is boiled with dilute hydrochloric acid, with exclusion of air, finally evaporated to dryness, and the residue again extracted with alcohol. This new alcoholic solution (D) can be divided by acetate and subacetate of lead into two principal portions.

By operating in this manner the complex products of putrefaction are readily separated into four portions.

GAUTIER has also employed the following method: The putrid liquids, after the removal of fats, are feebly acidified with very dilute sulphuric acid, then distilled in *vacuo* at a low temperature. The distillate contains ammonia, phenol, indol, and skatol. The syrupy residue, separated from any crystals which may have formed, is rendered alkaline with baryta, filtered, and extracted a great number of times with chloroform, in order to dissolve the bases. The solution is distilled at a low temperature, either in *vacuo* or in a current of carbonic acid. The contents of the retort, on being treated with water and tartaric acid, separate into a brown resin and a liquid portion. The latter is removed and treated with a dilute solution of potash, when it gives off the odor of carbylamin, which was discovered by GAUTIER in 1866, and which, according to CALMEL, is a constituent of the venom of toads. The alkali also sets free the bases, which are removed by extraction with ether, and the ether evaporated in a current of carbonic acid gas under slight pressure, then under a bell-jar over caustic potash. The bases may be separated by fractional

precipitation with platinum chlorid, or, if present in sufficient quantity, by distillation *in vacuo*.

GAUTIER has in some instances modified his method as follows: The alkaline putrid liquid is treated with oxalic acid (instead of sulphuric acid) to free acidulation and as long as the fatty acids continue to separate. The liquid is then warmed and distilled as long as a turbid fluid passes over. Pyrrol, skatol, phenol, indol, volatile fatty acids, and some of the ammonia pass over. The portion which remains in the retort is rendered alkaline with lime-water. The precipitate which forms, and which contains the greater part of the fixed fatty acids, is removed. The liquid portion, which is alkaline, is distilled to dryness, care being taken to receive the distillate in very dilute sulphuric acid. The bases and ammonia pass over. The distillate is neutralized (with sulphuric acid) and evaporated almost to dryness, then decanted from ammonium sulphate, which crystallizes. The mother-liquor is extracted with concentrated alcohol, which dissolves the sulphates of the ptomaines. After driving off the alcohol the residue is rendered alkaline with caustic soda, and successively extracted with ether, petroleum-ether, and chloroform.

The lime-precipitate is dried and extracted with ether of thirty-six degrees, which removes any fixed bases that may be present.

REMARKS UPON THE METHODS.—The fundamental difference between the Stas-Otto and the Dragendorff methods consists in the fact that in the former the first extraction is made with a dilute solution of an organic acid (tartaric usually), while in the second a similar solution of a mineral acid (sulphuric) is employed. In their various modified forms any solvent may be used for separating the alkaloid from the other constituents of the original solution. Therefore, the question has been asked, Which is the more suitable acid for use in making the first solution? The answer to this question will also be the one to the question, Which is the better method of extracting ptomaines, the Stas-Otto method

or that of Dragendorff? The Italian chemists GUARESCHI and Mosso have attempted to answer this question experimentally, and the evidence which they have furnished is condemnatory of the method of Dragendorff. They show that basic bodies are formed by the action of the dilute sulphuric acid upon albuminous substances. As this point is of vital importance to the investigator in this branch of chemical science, we will give a brief abstract of the work of GUARESCHI and Mosso :

One kilogram of fresh meat was treated with dilute sulphuric acid (in the proportion recommended in the Dragendorff method) and alcohol. The dark solution after filtration was made alkaline with ammonium hydrate and extracted with ether. The ethereal solution gave on evaporation an oily substance which had the odor of extracts obtained from putrid fibrin. This substance, which was obtained in considerable quantity, was soluble in water and strongly alkaline in reaction. After neutralization with hydrochloric acid, its aqueous solutions gave the following alkaloidal tests :

1. With platinum chlorid, a yellowish-red precipitate, insoluble in water, alcohol, and ether, and apparently identical with the compound obtained from putrid fibrin with the same reagent.

2. With gold chlorid, yellow precipitate, then reduction to metallic gold.

3. With phosphomolybdic acid, a heavy, yellow precipitate, forming a blue solution on the addition of ammonium hydrate.

4. With phosphotungstic acid, a white precipitate.

5. With Mayer's reagent, a heavy, whitish precipitate.

6. With picric acid, white precipitate, instantly.

7. With iodine in potassium iodid solution, a heavy kermes-red precipitate.

8. With tannic acid, white precipitate.

9. With mercuric chlorid, white, amorphous precipitate.

10. With Marmé's reagent, heavy precipitate.

11. With potassium ferricyanid, no precipitate, but a

cloudiness, with a formation of Prussian-blue on the addition of ferric chlorid.

The same quantity of this meat was also treated by the Stas-Otto method. The alcoholic extract was evaporated on the water-bath, and not in vacuo. The acid was neutralized with sodium bicarbonate. The ether-extract gave on evaporation a faintly yellow residue, of not unpleasant odor and feebly alkaline reaction. After neutralization with hydrochloric acid, it was only slightly soluble in water. The pale yellow filtrate gave no precipitate with Nos. 1, 2, 8, 9, and 10 of the above-mentioned reagents, but gave a slight turbidity with Nos. 3, 4, 5, 6, and 7, and with 11 formed Prussian-blue.

GUARESCHI and MOSSO conclude from this and other experiments that the Dragendorff method is not suitable for the extraction of ptomaines, and they recommend the employment of the Stas-Otto method with these conditions: (1) no more acid should be added than is absolutely necessary to keep the reaction acid; (2) the heat used in evaporation should not be great, and it is better that evaporation should be made in vacuo. In this way, they say, no ptomain will be obtained from fresh tissue.

The same investigators extracted fresh flesh without the addition of any acid. Thirty kilograms of perfectly fresh meat were digested for two hours at from 50° to 60° with about one and one-half volumes of water. The fluids of the meat contained enough acid to give to the whole of this solution an acid reaction. It was evaporated to half its volume on the water-bath, filtered, and evaporated still further. The small residue was taken up with about four volumes of 96 per cent. alcohol. The reddish, alcoholic solution left on evaporation on the water-bath a brownish residue, which was dissolved in water and extracted with ether (A), then the solution was made alkaline with ammonium hydrate and again extracted with ether (B).

A gave on evaporation and cooling crystals of methylhydantoin, while the mother-liquor contained acetic acid.

B also yielded crystals of methyl-hydantoin, while the mother-liquor gave alkaloidal reactions with most of the general alkaloidal reagents, none with platinum chlorid. Methyl-hydantoin does not give these reactions.

MARINO-ZUCO has made many comparative tests with these two methods. He ascertained that by treating fresh eggs, brain, liver, spleen, kidney, lungs, heart, and blood by either of the methods, he could obtain a substance which gave alkaloidal reactions, and which he demonstrated to be cholin. His experiments led him to believe that cholin did not exist preformed in these fresh tissues, but that it resulted from the action of the dilute acids upon lecithin. It was found most abundantly in those tissues which are rich in lecithin, such as the yolks of eggs, brain, liver, and blood; while only traces could be obtained from the whites of eggs, lungs, and heart. The method of Dragendorff was found to furnish much larger quantities of cholin than could be obtained by the Stas-Otto method.

COPPOLA agrees with his countrymen, mentioned above, in condemning the method of Dragendorff.

Enough has been said to show that results obtained by the Stas-Otto method are much more reliable than those secured by the method of Dragendorff. However, the former is not a perfect method, nor has a perfect one yet been devised. The principal difficulties met with in the Stas-Otto method are as follows:

1. In most instances the extraction of the base is very incomplete.
2. The degree to which the putrefactive alkaloid is removed by the solvent will depend very largely upon the nature of the other substances present. This fact in some cases aids and in others hinders the labors of the investigator. Thus, several ptomaines, which when pure are wholly insoluble in ether, may be removed, in part at least, from organic mixtures by this solvent by passing into the solution along with other substances; but if the attempt is made to purify one of these bases by repeated solution and extraction with ether, the result is a failure, because the more perfectly

the alkaloid is freed from impurities, the less soluble it is in ether. This criticism, however, is equally applicable to the Dragendorff method, and to all others in so far as extractions are made.

However, we may state that whenever it is applicable this method is the best now employed. By it the substances are submitted to the least chemical manipulation, and the results obtained are the most reliable. Many of the more complex putrefactive products are so easily decomposed or otherwise altered that the investigator should seek to isolate them by the simplest methods possible. If it can be done without the addition of any acid or without the application of heat, so much the better.

Especially is the modification of this method employed by MARINO-ZUCCO, and already described, to be commended.

By his method BRIEGER has discovered a considerable number of basic bodies, and has given great impetus to the study of the chemistry of putrefaction. The method is capable of a great many modifications. As long ago as 1868, BERGMANN and SCHMIEDEBERG employed precipitation with metallic salts in order to obtain sepsin from putrid yeast. The method used by them was as follows: Putrid yeast was diffused through parchment-paper; the diffusate was acidified with hydrochloric acid, and treated with mercuric chlorid solution until a heavy cloudiness and, after some time, a slight precipitate formed. This was removed by filtration; the filtrate was rendered strongly alkaline with sodium carbonate, and then further treated with a solution of mercuric chlorid as long as a precipitate formed. This precipitate was collected on a filter, washed, suspended in a little acidified water, and decomposed with hydrogen sulphid. The precipitate was removed, the free hydrochloric acid in the filtrate taken up with silver carbonate, and the excess of silver removed with hydrogen sulphid. The filtrate was evaporated to dryness; the residue dissolved in alcohol (a part remaining insoluble), and acidified with sulphuric acid, when a colorless or slightly yel-

low crystalline precipitate formed. The crystalline sepsin sulphate was purified by solution in water and precipitation with alcohol.

BRIEGER has obtained some of his bases by a much simplified modification of his complete method, which we have given in full. For instance, in obtaining neuridin, he treated the aqueous extract of the putrid material, after boiling and filtration, with mercuric chlorid, collected the precipitate, decomposed it with hydrogen sulphid, evaporated the filtrate on the water-bath, and extracted the base from the residue with dilute alcohol.

By this method and its modifications BRIEGER has obtained many brilliant results, among which may be mentioned his discovery of mytilotoxin, typhotoxin, and tetanin. However, the method is not free from criticism. The great number of chemical manipulations to which the organic matter is subjected is liable to lead to the formation of some basic substances and to the destruction of others. One is justified in considering the isolated base as pre-existing in the original material only when it produces symptoms identical with those caused by the substance from which it is extracted. There can be no doubt that by this method many ptomaïns would be decomposed. With it EHRENBURG obtained from poisonous sausage only inert bases, and tyrotoxicon, the ptomaïn of poisonous cheese, is decomposed both by heat and the hydrogen sulphid employed. The origin of the ptomaïns possessing a muscarine-like action discovered by BRIEGER has been questioned by GRAM, who states that when the lactate of cholin, an inert substance which is widely distributed both in plants and animals, is heated, it is converted into a poison with such an action. This, however, has been effectually disproven, as will be seen in a subsequent chapter.

CHAPTER X.

METHODS OF ISOLATING THE TOXINS.

HANKIN employed the following process in preparing his anthrax-proteid :

“The cultures are made in 0.1 per cent. Liebig’s extract of meat solution, to which some fibrin is added. The Liebig’s extract is very difficult to sterilize, and must be heated for two or three hours in the steam sterilizer on two or three successive days. The fibrin must be added only after this has been done, and then the flask is re-sterilized by repeated heating to boiling-point, for a short time only on each occasion. If the fibrin were added at first, it would be decomposed by the prolonged boiling. By the above method this only occurs to a slight degree, a mere trace of pepton being present in the sterilized culture-fluid. After sterilizing, this is inoculated with the blood of an animal dead of anthrax, and kept at the ordinary temperature. The anthrax forms a typical growth on the masses of fibrin, and samples of the liquid removed on successive days show a gradual increase in the strength of their biuret-reaction. After about a week the liquid is filtered and the albumose extracted. The reason for not keeping the flask at a temperature of 37° is that the albumose is gradually decomposed into pepton by the anthrax-ferment present, and this change takes place more rapidly at the higher temperature. For instance, I have found scarcely a trace of albumose in a culture which had been kept at 37° for a week, and which gave a strong biuret-reaction. The albumose is separated from the culture-liquid thus prepared by saturation with ammonium sulphate. It is better to acidulate it slightly by adding a little acetic acid. The bulky precipitate of albumose which then appears is filtered off, and the salt separated from

it by dialysis. An excess of thymol must be added at this stage to prevent putrefaction, or the dialysis can be carried on in a current of water which is warmed to from 45° to 50° , at which temperature the growth of microorganisms is inhibited. After dialyzing for twenty-four hours or more the greater part of the salt will have vanished, and the albumose will be found in solution in a considerable quantity of water which will not have passed through the parchment. It is now necessary merely to concentrate the solution and precipitate the albumose by the addition of alcohol. In my earlier experiments this was accomplished by evaporating *in vacuo* at a temperature of 45° to 48° . When at length the liquid has been reduced to a few cubic centimetres it is poured into alcohol, and the precipitated albumose is filtered off, washed with the same reagent (alcohol), and dried.

"Evaporating *in vacuo* is a long and tedious process, and it requires a somewhat complicated apparatus. When it is used for pathogenic albumoses there is always a risk of the temperature employed destroying or diminishing their physiological properties. Further, if the albumose is allowed to evaporate to dryness, it may be difficult to make it pass into solution again. To avoid these difficulties I have designed a method of concentrating such solutions which is less objectionable. It depends on the principle that, if alcohol and water are placed on opposite sides of a membrane, the water rapidly dialyzes through to mix with the alcohol, while only traces of alcohol pass through to mix with the water. Consequently, if a watery solution of albumoses is dialyzed against alcohol, the solution diminishes in bulk and is rapidly concentrated, owing to the passage of the water through the membrane.

"My *modus operandi* is to place the dilute albumose-solution in a parchment sausage skin which is immersed in a foot-glass full of methylated spirit. The spirit can be changed after some hours if it is desired to prolong the process; but this is not usually necessary. In this way I have been able to bring 400 c.c. of albumose-solution down to 100 c.c. in the course of a single night, at the ordinary temperature, without risk to

the albumose or trouble to myself. The concentrated solution is then poured into absolute alcohol, which precipitates the albumose and removes any impurities that might be derived from the methylated spirit. This prolonged treatment with alcohol will tend to remove any free ptomaines or other substances soluble in alcohol. Peptons and salts present in the culture-liquid remained for the most part in solution when the albumose was precipitated with $(\text{NH}_4)_2\text{SO}_4$. No soluble proteids (except traces of pepton) were present in the culture-medium."

MARMIER isolates the anthrax-toxin found in his culture-fluid, mentioned on page 155, in the following manner: The culture is filtered and saturated with ammonium sulphate at ordinary temperature. After standing for some hours the precipitated toxin is collected on a filter and washed with a saturated solution of ammonium sulphate. The precipitate is then dissolved in water, freed from ammonium sulphate by dialysis, concentrated over sulphuric acid, precipitated with strong alcohol, washed with absolute alcohol, and dried.

The toxins obtained by these methods are far from pure. They are carried down mechanically by the precipitation of the proteid with ammonium sulphate.

BRIEGER states that the toxin of the most virulent tetanus-cultures is not precipitated by ammonium sulphate. He has employed neutral lead acetate, 5 grams to 100 c.c. of the culture. All the toxin is carried down in this precipitation. The precipitate is then shaken with sodium sulphate, whereby the tetanus-poison is set free.

CHAPTER XI.

THE IMPORTANCE OF BACTERIAL PRODUCTS TO THE TOXICOLOGIST.

THE presence in the cadaver of substances which give not only the general alkaloidal reactions, but respond to some of the tests which have hitherto been considered characteristic of individual vegetable alkaloids, must be of the greatest importance to toxicologists. The possibility of mistaking putrefactive for vegetable alkaloids should always be borne in mind by the chemist in making his medico-legal investigations. On the other hand, as we have seen in preceding chapters, cases of poisoning by ptomaines sometimes terminate fatally, and in such instances the chemist should not be satisfied with determining the absence of mineral and vegetable poisons, but should strive to detect in the food or in the dead body positive evidence of the presence of the putrefactive alkaloid.

We will give a brief account of those cases in which putrefactive substances have been found to resemble in their reactions the vegetable alkaloids.

CONIIN-LIKE SUBSTANCES.—The most celebrated case in which a substance giving reactions similar to those of coniin has been found was the Brandes-Krebs trial, which took place in Braunschweig in 1874. From the undecomposed parts of the body two chemists obtained, in addition to arsenic, an alkaloid which they pronounced coniin. This substance was referred to Otto for further examination. Otto reported that the substance was neither coniin nor nicotin, nor any vegetable alkaloid with which he was acquainted. Otto converted the substance into an oxalate, dissolved it in alcohol, evaporated the alcohol, dissolved the residue in water, ren-

dered this solution alkaline with potash, then extracted the base with petroleum-ether. On evaporation of the petroleum-ether the alkaloid appeared as a bright-yellow oil, which had a strong, unpleasant odor, quite different, however, from that of coniin. It was strongly alkaline and had an intensely bitter taste. At ordinary temperature it was volatile. From its aqueous solution it was precipitated by the chlorids of gold, platinum, and mercury. In these reactions it resembled nicotine, from which it differed in the double refractive and crystalline character of its hydrochlorid. With an ethereal solution of iodine this substance did not give the Roussin-test for nicotine, but instead of the long ruby-red crystals there appeared small, dark-green, needle-shaped crystals.

This substance was found to be highly poisonous. Seven centigrams injected subcutaneously into a large frog produced instantaneous death, and forty-four milligrams given to a pigeon caused a similar result. On account of its poisonous properties the jury of medical experts decided that the substance was a vegetable alkaloid. OTTO says that this decision astounded the chemists.

BROUARDEL and BOUTMY found in the body of a woman who had died, after suffering, with ten other persons, from choleraic symptoms from eating of a stuffed goose, a base which gave the odor of coniin and the same reactions with gold chlorid and iodine in potassium iodid, etc., as coniin. The same base was found in the remainder of the goose. But it did not give a red coloration with the vapor of hydrochloric acid, and it did not form butyric acid on oxidation, and, although it was poisonous, it did not produce in frogs the symptoms of coniin-poisoning.

SELMÉ repeatedly found coniin-like substances in decomposing animal tissue. By distilling an alcoholic extract from a cadaver, acidifying the distillate with hydrochloric acid, evaporating, treating the residue with barium hydrate and ether, and allowing the ether to evaporate spontaneously, he obtained a residue of volatile bases, the greater part of which consisted of trimethylamin. After removing the trimethy-

lamin the residue had the odor of the urine of the mouse. Later, SELMI obtained an unmistakable coniin-odor from a chloroform-extract of the viscera of a person who had been buried six months, and in another case ten months after burial. Two or three drops of an aqueous solution of the alkaline residue of the chloroform-extract allowed to evaporate on a glass plate gave off such a penetrating odor that SELMI was compelled to withdraw from close proximity to the substance. The odor imparted to his hands in testing the substance with the general alkaloidal reagents remained for half an hour. This volatile base seemed to be formed by the spontaneous decomposition of other ptomaines.

An aqueous solution of a ptomain obtained by SELMI by extraction with ether, according to the Stas-Otto method, from the undecomposed parts of a cadaver, had no marked odor, but after being kept for a long time in a sealed tube, it not only gave off a marked coniin-odor, but the vapor turned red litmus-paper blue. Again, the sulphate of a ptomain obtained from putrid egg-albumin, on standing, formed in two layers, one of which was a golden-yellow liquid, which on being treated with barium hydrate gave off ammonia, and later the odor of coniin. Since butyric and acetic acids were formed by the oxidation of this base, SELMI concluded that he had real coniin or methyleconiin, and that it was formed by the oxidation of certain fixed ptomaines, or by the action of different amido-bases on volatile fatty acids. Therefore SELMI believed in the spontaneous origin of coniin or closely allied bases in putrid matter, also in the existence of a "cadaveric coniin."

The substance which was found by SONNENSCHNIG in a criminal trial in East Prussia, and which was believed by that chemist to be the alkaloid of the water-hemlock (*Cicuta virosa*), is thought by OTTO, HUSEMANN, and others, to be a cadaveric coniin. OTTO says that the symptoms reported in the case were not those of either coniin or cicuta. SONNENSCHNIG obtained the base six weeks after the exhumation of the body, which had been buried three months. The base

had the odor of coniin, the taste of tobacco, gave with potassium bichromate and sulphuric acid the odor of butyric acid, and behaved with reagents like couiin.

HUSEMANN states that at present it is very difficult, if not impossible, for the chemist to state with certainty that he has detected true coniin in the dead body. The symptoms and the post-mortem appearances must conform with those induced by the vegetable alkaloid. The analysis must be made before decomposition sets in, and the amount of the base found must be sufficient for physiological experiments to be made with it.

A NICOTIN-LIKE SUBSTANCE.—WOLCKENHAAR obtained from the decomposed intestines of a woman, who had been dead six weeks, by extraction with ether from an alkaline solution, a base which bore a close resemblance to nicotine. The base was fluid, at first yellow, but on being exposed to the air brownish-yellow. It was strongly alkaline in reaction and gave off an odor resembling nicotine, but stronger, not ethereal, benumbing and similar to that of fresh poppy-heads. It was soluble in all proportions in water, and the solutions, which did not become cloudy on the application of heat, did not taste bitter, but were slightly pungent. The peculiar odor did not disappear on saturating the base with oxalic acid. The hydrochlorid was yellow, like varnish, had a strong odor, and became moist on exposure to the air. Under the microscope it showed no crystals, differing in this respect from nicotine hydrochlorid. It differed from nicotine also in its reactions with potassio-bismuthic iodid, gold chlorid, iodine solution, mercuric chlorid, and platinum chlorid. It also failed to give the Roussin-test for nicotine. Moreover, it could not be identified with trimethylamin, spartein, mercurialin, lobelin, or other fluid and volatile bases.

The studies of RÖRSCH and FASSBENDER (page 33), of SCHWANERT (page 33), of LIEBERMANN (page 35), and of SELMI (page 36), have already been referred to in a preceding chapter.

STRYCHNIN-LIKE SUBSTANCES.—In a criminal prosecution at Verona, CIOTTA obtained from the exhumed, but only slightly decomposed body, an alkaloid which gave a crystalline precipitate with iodine in hydriodic acid, a red coloration with hydriodic acid, and a color-test similar to that of strychnin with sulphuric acid and potassium bichromate, and with other oxidizing agents. This substance was strongly poisonous, but did not produce the tetanic convulsions which are characteristic of strychnin. CIOTTA pronounced this substance as probably identical with strychnin. Portions of the body were subsequently submitted to SELMI for his opinion. SELMI found that the substance which gave the color-reaction was not crystalline, and that there was only “the presumption of a bitter taste to it,” while one part of strychnin in 40,000 parts of water is intensely bitter. SELMI also held that many ptomaines give reactions similar to strychnin with iodine in hydriodic acid and with hydriodic acid. He also held that its physiological properties were such that it could not be strychnin. This substance could hardly have been aspidospermin, which reacts with sulphuric acid and potassium bichromate similarly to strychnin, because quebracho-bark, in which this alkaloid is found, was not at that time used as a medicine or known in Italy.

Ptomaines giving reactions similar to those of strychnin, and also causing tetanic spasms, have been found in Italy in decomposed corn-meal. SELMI obtained one of these substances, but found that it differed from strychnin inasmuch as it could not be extracted with ether.

LOMBROSO has named the poisonous substance found in decomposed corn-meal pellagroecin, but this is really a mixture of ptomaines, some of which produce narcosis and paralysis, and others produce the symptoms of nicotine-poisoning instead of the spasms caused by strychnin.

A MORPHIN-LIKE SUBSTANCE.—In the Sonzogna trial, at Cremona, Italy, the experts seem to have confounded a ptomaine with morphine. This substance was not removed from

either alkaline or acid solutions with ether, but could be extracted with amyl alcohol. It reduced iodic acid, but in its other reactions, as well as in its physiological properties, it bore no resemblance to morphin. In frogs it arrested the heart in systole, which is said never to happen in poisoning with morphin. It failed to give both the ferric chlorid and the Pellagri test for morphin.

In the same body there was found a substance which was extracted from alkaline solutions with ether, and which gave, with hydrochloric acid and a few drops of sulphuric acid, on the application of heat, a reddish residue similar to that obtained by the same reagents with codein, but in its other reactions it did not resemble this alkaloid.

Many of the tests for morphin employed by toxicologists are fallacious. In the examination of a stomach, and part of a liver, sent from Lincoln, Neb., VAUGHAN, following the method of Dragendorff, obtained in the amylie alcohol extract from alkaline solution a residue that gave with more or less distinctness all of the principal color-tests for morphin; but failing to obtain crystals that could be identified as those of this alkaloid, the absence of morphin was reported. HAINES, working with the same material, obtained similar reactions, but he also was unable to secure the crystals, and made a negative report. Afterward, it was quite positively shown that death had been caused in this case by a blow on the back of the head with a heavy piece of iron.

In the Buchanan case in New York, the symptoms as sworn to by the attending physician clearly were not those of morphin, and all the tests obtained by the experts were duplicated with putrefactive products.

These cases induced VAUGHAN to make some experimental studies, which are reported in Hamilton's *System of Legal Medicine* as follows :

The above-mentioned facts induced the writer to undertake some experimental studies upon this point. In this work the author has been greatly aided by one of his students E. M. Houghton. The results which we obtained are sufficient to

convince us that the identification of morphin in the liver and other organs in cases of suspected poisoning is beset with difficulties not provided for by the methods now generally employed.

Since the substances which vitiate the morphin-tests are of bacterial origin, and since bacterial products vary with the conditions under which the germs producing them grow, it is essential that the putrefactive changes which the tissue undergoes before the tests are begun should occur under those conditions, as nearly as possible, which exist in the cadaver. Neglect of this point has undoubtedly been the chief factor in securing the confidence of toxicologists, generally in the methods of Dragendorff and Stas-Otto. Many most skillful chemists have carried companion-portions of decomposed tissue, one portion with, and the other without morphin, through the process of extraction recommended by Dragendorff, and have obtained satisfactory results, finding that the proper residue responds to the color-tests in the one instance, and fails to do so in the other. Tissues have been thus tested in apparently every stage of putrefaction, and yet the results have been satisfactory and confirmatory of the methods now generally employed. There is one point, however, which has been constantly overlooked. The putrefaction to which the tissues in these experiments are subjected has been aërobie, while that occurring in the dead body is anaërobie; consequently the putrefactive products are not the same in the two cases. This leads us to state that in all experimental studies of the value of the tests for morphin in decomposing tissue, the decomposition must be allowed to proceed in the absence of oxygen. This is the first point. The second is probably of equal importance, and this concerns the kind of tissue employed. The upper portion of the small intestine (and the adjacent tissue after death) has a bacterial flora peculiar to itself. These tissues are the ones quite universally examined in medico-legal cases, and consist of the small intestine itself, the stomach, the liver, the pancreas, the spleen, and, in some instances, the kidneys. Of

course, the bacteria present in the small intestine during life may after death extend to all the abdominal and thoracic viscera. Since the liver is so generally examined, we decided to ascertain the effects, if any, of the putrefactive products formed in this organ, decomposing under anaërobie conditions, on the tests for morphin carried out according to the scheme of Dragendorff. Recognizing the fact that arsenic is so frequently employed in the form of an embalming-fluid, it was thought best to add this to the liver. The experiment is detailed in the following statement :

Five kilograms of ox-liver chopped finely and mixed with two grams of arsenic dissolved in caustic potash were placed in a large bottle. The bottle was closed with a cork and sealed with paraffin. A glass-tube bent at a right-angle was inserted in the centre of the cork, while the other end of the tube was connected by means of a short piece of rubber-tubing with a Drechsel wash-bottle. The other arm of the wash-bottle was connected with a receiver filled with water. The rubber connecting the large bottle with the wash-bottle was supplied with a clamp.

During the first fifteen or twenty days this clamp was left open, and a large amount of gas passed through the wash-bottle and collected in the receiver. After the above-mentioned time, which varies according to temperature, the passage of gas ceases, and the water rises in the receiver, absorbing the collected gas. When this occurred the bottle containing the tissue and the wash-bottle were disconnected, and the clamp on the rubber-tubing was closed. By this time the chopped liver has become sufficiently fluid to absorb the gas as fast as it is formed, and unless the bottles are disconnected the water in the wash-bottle may be drawn back into the large bottle.

The fermentation was allowed to continue for thirty days, counting from the beginning. Then the contents of the bottle, decidedly acid in reaction, and giving off a not disagreeable ethereal odor, were poured into a large dish. A considerable portion of the tissue had become fluid by this time.

One kilogram of this decomposed tissue was placed in each of three evaporating-dishes, and these were marked A, B, and C. To B, 130 milligrams of morphin sulphate were added, and to C the same amount of morphin, together with 0.5 gram of indol, skatol, and phenol. No addition was made to A. These portions were carried through the manipulations recommended by DRAGENDORFF (*Die gerichtlich-chemische Ermittlung von Giften, dritte Auflage*, 1888).

To each 100 c.c. of the fluid 5 c.c. of dilute (1 : 5 sulphuric) acid were added. Then 500 c.c. of distilled water were added to each dish, and these were kept at from 40° to 50° for eight hours.

Next, each portion was filtered through a plaited filter (No. 572 of Schleicher & Schull). The fluid passed through quickly, and formed a clear, brownish filtrate. The filtrates were evaporated at 50° to 600 c.c., and four volumes of absolute alcohol were added to each portion. These mixtures were allowed to stand for twelve hours, and in each a brown resinous precipitate formed. After filtration the alcohol was removed by distillation. A fatty-like residue formed in each flask on the removal of the alcohol, and this was removed by filtration.

The acid solutions were then thoroughly shaken, each with four volumes of petroleum-ether. The ethereal layers, when drawn off and evaporated in portions, left very slight residue.

The residues from A and B gave no reaction on the application of the color-tests for morphin mentioned below.

The residue from C showed minute traces of indol with nitric acid alone, and with sulphuric acid containing nitric.

The acid solutions were next shaken with benzol. The benzol-residues gave no response to the morphin-tests.

Chloroform was then applied as a solvent. The residue in this case gave none of the reactions.

The acid solutions were now rendered alkaline with ammonium hydrate, and shaken successively with petroleum-ether, benzol, and chloroform. None of the residues from these solvents responded to the morphin-test.

The alkaline solutions, having been subjected to the above-mentioned processes of purification, were shaken, each with five volumes of amyllic alcohol. The shaking was frequently repeated during the afternoon, and then the mixtures were placed in separators and allowed to stand for eighteen hours. The amyllic alcohol extracts (A, B, C) evaporated on the water-bath gave the following reactions:

Reagents.	A, B, C.
Nitric acid	All gave a lemon-brown color.
Sulphuric acid	None showed any change.
Sulphuric with nitric acid	All gave a lemon-yellow, slowly changing to a pink.
Ferric chlorid	All gave a dirty green.
Iodic acid	All promptly reduced the iodic acid.
Fröhde's reagent	All gave a blue color, without any violet.
Sulphuric acid and cane-sugar	All became brownish-red, changing to a wine-red.

Portions of the amyllic alcohol extract allowed to evaporate spontaneously showed the same reactions as those given above.

The remaining portions of the amyllic alcohol solutions were now shaken with distilled water acidified with sulphuric acid. After separation, portions of the amyllic alcohol were evaporated and subjected to the above-mentioned tests, with negative results in each case. This shows that amyllic alcohol does not dissolve from acid solutions the substance or substances interfering with the morphin-test.

The acid aqueous solutions of A, B, and C were again rendered alkaline with ammonium hydrate, and shaken with amyllic alcohol. The residues from these amyllic alcohol extracts were evaporated and subjected to the following tests:

Reagents.	A, B, C.
Nitric acid	All became lemon-yellow.
Sulphuric acid	No change in any.
Sulphuric acid with nitric acid	All became lemon-yellow.
Ferric chlorid	All became bluish-green.
Iodic acid	All promptly reduced iodic acid.
Fröhde's reagent	All became blue, with a faint and evanescent purple in B and C.
Pellagri's reagent	All responded promptly.

The above-mentioned experiment, which has been repeated with no variation in results, convinces us that the tests for

morphin by following the scheme of Dragendorff are altogether untrustworthy. Naturally the question arises, What is the nature of the substance or substances which give these color-reactions? Quite as naturally the answer that these substances consist of indol and its derivatives suggests itself. The probabilities in favor of this answer may be briefly stated as follows:

1. Germs which produce indol and its derivatives are native, and, so far as we know, constant representatives of the bacterial flora of the upper portion of the small intestine. There are many indol-forming germs, and while some of these may be present in any tissue, they are certainly present, in health and in disease, during life and after death, in the small intestine.

2. Indol and its derivatives are products of anaërobic putrefaction, and this accounts for the fact that the reactions which we obtained are not familiar to those toxicologists who have experimented with tissue allowed to putrefy in the presence of oxygen. The apparatus which we used in our experimental work is practically the same as that employed by E. and H. Salkowski (*Zeitschrift f. physiologische Chemie*, B. 8, S. 462) in the preparation of indol. Moreover, in the preparation of indol the same peculiarity in the evolution of gas is observed as in our work.

It was on account of our belief that indol and its derivatives had been in some instances mistaken for morphin that we were led to add these substances to C in our experiment.

We have obtained several samples of indol and skatol, and have compared the reactions obtained with these on the application of the color-tests for morphin.

The samples of indol may be briefly described as follows:

- No. 1. Prepared by myself from decomposing pancreas. It is a brown, granular substance, and is probably not chemically pure. This fact, however, does not unfit this sample for experiments on the point under consideration, because any impurities which it may contain originated in the decomposing

tissue, and may be present in the same substance obtained from like tissue.

No. 2. Obtained from Merck. The order was simply for "indol," without any specifications whether it should be synthetic or putrefactive. It is brownish-red in color.

No. 3. Obtained from Schuchardt and ordered as synthetic indol, which it undoubtedly is. This sample is white and in flakes.

No. 4. Obtained from Kahlbaum. Putrefactive indol was ordered, and the label is simply "indol." This sample consists of white flakes.

These samples were submitted to the following tests:

Reagent.	No. 1.	No. 2.	No. 3.
Nitric acid.	Bluish-black with violet border.	Reddish-brown.	Reddish-brown.
Sulphuric acid.	Yellowish-green.	Brown.	Greenish-yellow.
Sulphuric acid with nitric acid.	Same as with nitric acid alone.		
Ferrie chlorid.	No change at first, but all become greenish-blue.		
Iodic acid.	No reduction.		
Fröhde's reagent.	Reddish, then dark blue.	Reddish, then greenish-blue.	Reddish, then greenish-blue.

Reagent.	No. 4.	Pure morphin sulphate.
Nitric acid.	Reddish-brown.	Brownish-red, passing into lemon-yellow.
Sulphuric acid.	Brownish-red.	Faint yellow.
Sulphuric acid with nitric acid.		Brownish-red.
Ferrie chlorid.		Blue.
Iodic acid.		Reduced.
Fröhde's reagent.	Reddish, then greenish-blue.	Purple, then blue.

Two samples of skatol (No. 1 from Schuchardt and No. 2 from Kahlbaum) were compared with morphin with the following results:

Reagent.	No. 1.	No. 2.	Morphin sulphate.
Nitric acid.	All become lemon-yellow.		
Sulphuric acid.	All become very faintly yellow.		
Sulphuric acid with nitric acid.	All become more of a red than with nitric acid alone.		
Ferrie chlorid.	No change.	No change.	Blue.
Fröhde's reagent.	Green to blue.	Green to blue.	Purple to blue.
Iodic acid.	All promptly reduce the acid.		

While it would be comparatively easy to distinguish pure morphin from either indol or skatol, it must be admitted, from the results of the experiments already detailed, that the separation of morphin from tissue, decomposing in the absence of oxygen, and its identification, are, by the methods now generally employed, so uncertain that the conscientious chemist will seek for methods free from these sources of error before he gives positive testimony of the presence of this alkaloid.

I have spoken of indol and its derivatives as being present in the decomposing tissue, and it should be stated that the number of known indol-derivatives is by no means small, and how many others there may be which remain unknown, no one can tell. Many of these substances give brilliant color-reactions. Indol was first obtained by *BAEYER* by the reduction of indigo. Later, *KÜHN* and *NESCKI* independently obtained indol with skatol by the putrefaction of albuminous substances.

There has been some difference of opinion as to the identity of the indol obtained by putrefaction and that which results from the reduction of indigo. According to *BAUMANN*, neither indol nor skatol originates directly from proteids, but both arise from the decomposition of a substance soluble in ether containing alcohol. Skatol is methyl indol.

Indoxyl is an easily decomposable substance, which gives some striking color-reactions, among which may be mentioned the production of indigo-blue with ferric chlorid in the presence of free hydrochloric acid. Skatol-carbonic acid is another product of putrefaction, *E.* and *H. SALKOWSKI* having ob-

tained 1.3 grams from 2 kilograms of moist fibrin after twenty-six days' putrefaction. Among the known color-reactions of this substance, HOPPE-SEYLER mentions the following:

1. If a dilute solution of this acid (1 : 1000) be treated with a few drops of pure hydrochloric acid of 1.2 specific gravity, and then with a few drops of potassium nitrate solution (2 per cent.), a cherry-red coloration is produced, and later a red precipitate falls.

2. If such a solution be mixed with an equal volume of hydrochloric acid, and then a few drops of chlorid of lime solution ($\frac{1}{2}$ per cent.) be added, a purple-red color is produced.

3. Treated with a few drops of hydrochloric acid, then with two or three drops of a very dilute solution of ferric chlorid, and heated, the mixture becomes intensely violet before boiling, Skatol-carbonic acid is non-volatile.

Skatol acetic acid has been obtained by NENCKI by the anaërobic putrefaction of serum-albumin. The aqueous solutions of this substance give with ferric chlorid a white cloudiness, which on warming becomes a brick-red, and in more concentrated solution fire-red.

Both indigo-red and indigo-blue may be formed by the oxidation of indol.

Knowing now that indol and its derivatives are formed in anaërobic putrefaction, and that in Dragendorff's scheme for the separation and identification of vegetable alkaloids these substances appear in the residues which are tested for morphin, and knowing the great number and variety of color-reactions given by these substances, it may be asked how much reliance can be placed on the color-tests for morphin?

Besides the indol-bodies, certain other substances are formed in the anaërobic putrefaction of proteid substances. Among these are certain aromatic products of the putrefaction of tyrosin. The following may be mentioned:

1. Hydroparaacumaric acid (para-oxyphenyl-propionic acid). This substance gives with ferric chlorid a distinct, but evanescent, blue coloration.

2. Para-oxyphenyl-acetic acid. This substance gives with

ferrie chlorid a pale grayish-violet, which soon changes to a dirty green color.

Among other products of the anaërobic putrefaction of proteids phenol and parakresol may be mentioned.

Phenol gives with ferrie chlorid a violet color.

Parakresol gives with ferrie chlorid a blue coloration.

With the above-mentioned substances in a decomposing liver, and knowing that some of them at least are present in the amylic alcohol residue, following the process of Dragen-dorff, how much reliance, may again be asked, can be placed on the color.reactions of morphin? The conscientious chemist who swears that he will tell the truth, the whole truth, and nothing but the truth, may answer this question.

ATROPIN-LIKE SUBSTANCES.—Many investigators have found products of putrefaction which in their mydriatic properties resemble atropin and hyoseyamin. To this class belongs the substance observed by ZUELZER and SONNENSCHEIN. It was removed from alkaline solutions by ether, and formed microscopic crystals, an aqueous solution of which, when applied to the conjunctiva, produced a mydriatic effect, and, when administered internally, increased the action of the heart and arrested the movements of the intestines. Moreover, with certain alkaloidal reagents, such as platinum chlorid, it resembled atropin. But when heated with sulphuric acid and oxidizing agents it did not give the odor of blossoms (Reuss's test). However, SELMI found ptomatropins which with sulphuric acid and oxidizing agents did give the blossom-odor as distinctly as the vegetable atropin. These putrefactive bases also developed this odor spontaneously after standing for two or three days, and this does not happen with atropin. The odor was produced with the ptomatropins by nitric and sulphuric acids, both in the cold and on the application of heat, while these acids in the cold do not produce the odor with atropin.

Ptomatropins have been found in decomposing fish, corned beef, putrid game, and poisonous sausage. It is not known

whether there is only one or more of these poisons. The symptoms often resemble those of belladonna poisoning very closely. The throat becomes dry, the muscles of deglutition seem to be paralyzed, the secretion of perspiration and saliva is arrested, mydriasis may be marked, and there may be paralysis of accommodation, ptosis, and strabismus. In some instances delirium, and in others convulsions appear. The heart-beat becomes rapid and weak. The tongue is coated, and in the most dangerous cases constipation is obstinate. The general weakness may be extreme, and the voice wholly lost. Section shows the pharynx swollen, hemorrhagic spots in the œsophagus, stomach and intestines, cloudy swelling of the solitary follicles, and Peyer's patches and degeneration of the heart-muscles. The brain, lungs, and kidneys are often hyperæmic.

Extracts of putrid material will often cause more or less dilatation of the pupil in the lower animals when applied locally. The writer was recently appointed as one of a commission of two to inquire into the tests obtained by an expert who had reported four grains of atropin in the stomach of a man who had been dead for some weeks. The chief test relied upon by the chemist was that an ounce of an extract from the stomach dilated a cat's pupil about as much as a solution of four grains of atropin sulphate did. It is needless to comment on the validity of such evidence.

According to GIOTTO and SPICA, some ptomatropins give Vitali's reaction.

DIGITALIN-LIKE SUBSTANCES — Elsewhere we have referred to the discovery of a ptomaïn belonging to this class by RÖRSCH and FASBENDER (see page 33). TROTTARELLI obtained a similar substance from the brain of a man in whose abdominal viscera he could find no poison. The sulphate of this base gave on evaporation an aromatic-smelling and astringent-tasting residue. It became purple with sulphuric acid only, and dark red with hydrochloric and sulphuric acids. On frogs this ptomaïn showed no toxic effect.

A VERATRIN-LIKE SUBSTANCE.—BROUARDEL and BOUTMY obtained from a corpse which had lain in water for eighteen months, and a large portion of which had changed into adipocere, a ptomain resembling veratrin. It was removed from alkaline solutions by ether. On being heated with sulphuric acid it became violet. With a mixture of sulphuric acid and barium peroxide it became, in the cold, brick-red; and, on being heated, violet. With boiling hydrochloric acid it took on a cherry-red coloration. However, it differed from veratrin, inasmuch as it reduced ferric salts instantly, and when injected into frogs subcutaneously it did not induce in them the spasmodic muscular contractions characteristic of veratrin.

BECHAMP obtained by the Stas-Otto method from the products of the pancreatic digestion of fibrin an alkaloidal body which gave with sulphuric acid a beautiful carmin-red, similar to that given with gastric juice, and again extracting, he obtained a body which behaved with sulphuric acid similarly to curarin.

A DELPHININ-LIKE SUBSTANCE.—In 1870 General Gibbone, an Italian of prominence, died suddenly. His servant was accused of having poisoned him. Two chemists of some reputation reported the presence of delphinin in the viscera. It seemed somewhat improbable that the servant should know anything of so rare a substance, or that he should have been able to obtain it. However, two or more varieties of staphisagria grow in Southern Italy, and it was possible that the servant had used some preparation made by himself from the plant. The supposed alkaloid was given to SELMI, of Bologna, for further study. It was removed from alkaline solutions by ether. When heated with phosphoric acid it became red, and when brought in contact with concentrated sulphuric acid, reddish-brown. In these tests the substance resembled delphinin but with sulphuric acid and bromin-water, also with Fröhde's reagent, the colorations characteristic of the vegetable poison failed to appear. Moreover, SELMI showed

that delphinin gave the following reactions to which the suspected substance did not respond: 1. Delphinin dissolved in ether, and treated with a freshly prepared ethereal solution of platinic chlorid, gave a white, flocculent precipitate, which was insoluble in an equal volume of absolute alcohol. 2. Delphinin gave precipitates with anro-sodium hyposulphite, and with a sulphuric acid solution of cupro-sodium hyposulphite, the latter preeipitate being soluble in an excess of the reagent.

Finally, CIACCIA and VELLA showed that while delphinin arrests the heart of the frog in diastole, the suspected substance arrests it in systole.

A COLCHICIN-LIKE SUBSTANCE.—BAUMERT found in a case of suspected poisoning, twenty-two months after death, a substance which gave many of the reactions for colchicin. It was extracted from acid solutions with ether, to which it imparted a yellow color. On evaporation of the ether a yellow, amorphous substance remained, and this dissolved in warm water with yellow coloration. It could be extracted from acid solutions also by chloroform, benzol, and amylic alcohol, but not by petroleum-ether. It was removed with much more difficulty from alkaline solutions.

All the extracts were yellow, and left on evaporation a feebly alkaline, markedly bitter, sharp-tasting, amorphous yellow residue, which dissolved in water and dilute acids incompletely, forming a resin. When this resin was dissolved in dilute sodium hydrate, and the solution rendered acid by sulphuric acid, the same reactions were obtained as with the original extract.

With phosphomolybdic acid, phosphotungstic acid, potassio-bismuthic iodid, potassio-mercuric iodid, iodin in potassium iodid, tannic acid, and gold chlorid, this substance gave the same reactions which were obtained by parallel experiments with genuine colchicin; thus, the tannic acid precipitates were both soluble in alcohol, and the precipitates with phospho-

molybdic acid in both cases became blue on the addition of ammonium hydrate.

Concentrated sulphuric and dilute nitric and hydrochloric acids dissolved the supposed colchicin with yellow coloration. Strong nitric acid (1.4 sp. gr.) colored the substance dirty red, scarcely to be called a violet. When the substance was purified as much as possible, this color became a beautiful carmin-red. The addition of water changed the red into yellow, and caustic soda produced a dark, dirty orange.

In general, in the above-mentioned reactions, the putrefactive product agreed with the real colchicin, but the former gave precipitates with picric acid and platinum chlorid, while the latter gave no precipitates with these reagents.

In 1886, ZEISEL proposed the following test for colchicin: When a hydrochloric acid solution of the alkaloid is boiled with ferric chlorid, it becomes green, sometimes dark-green and cloudy. Now, if the fluid be agitated with chloroform, the chloroform will sink, taking up the coloring matter, and appearing brownish, granite-red, or dark, and the supernatant fluid clears up without becoming wholly colorless.

BAUMERT applied his test to both colchicin and the putrefactive product. To from two to five cubic centimetres of the suspected solution in a test-tube, he added from five to ten drops of strong hydrochloric acid and from four to six drops of a ten per cent. solution of ferric chlorid, then heated the mixture directly over a small flame until it was evaporated to half its volume or less. In the presence of one milligram of colchicin the originally bright-yellow solution became gradually olive-green, and, on further concentration, dark-green and cloudy. Then, on shaking the fluid with chloroform, admitting as much air as possible, the chloroform subsided, having a ruby-red color if as much as two milligrams of colchicin were present, and a bright-yellow if only one milligram, and the supernatant fluid became of a beautiful olive-green. When ether, petroleum-ether, benzol, carbon disulphid, or amylie alcohol was substituted for the chloroform, the coloration did not appear. From this BAUMERT infers that the red

coloring matter is either soluble in chloroform only, or that it is not formed until the chloroform is added.

BAUMERT found this test of great value in deciding whether or not the substance which he found was colchicin. The putrefactive product did not respond to the test.

Some of this substance was sent to BRIEGER, who decided that it was not a base, but a pepton-like substance. It was also found to be inert physiologically.

Before these investigations were made by BAUMERT, LIEBERMANN had found the same or a similar colchicin-like substance in the cadaver. His description differed from that of BAUMERT only in regard to the taste of the substance, LIEBERMANN having failed to observe any marked taste in the substance which he found, while, as has been stated, BAUMERT reported a distinctly bitter taste.

A colchicin-like substance has been found in beer, and it has been suggested that it was this which the above-mentioned toxicologists found in the bodies which they examined, but LIEBERMANN states that the man whose body he examined had been a total abstainer from beer.

TAMBA compared the reactions of ptomaines obtained from putrid sausage with similar reactions of various alkaloids, and then ascertained the effect upon the alkaloidal reactions by mixing alkaloids with the ptomaines. TAMBA's so-called ptomaines should be designated as extracts, and his results should not be accepted as conclusive. They are given here because they illustrate some of the difficulties met with in detecting the vegetable poisons in the presence of putrefactive products. They are as follows:

MORPHIN. Ptomaines are colored yellow with nitric acid; reddish-yellow with concentrated sulphuric acid; blue, violet, then green with Fröhde's reagent; yellow when evaporated with concentrated sulphuric acid, then treated with hydrochloric acid and decomposed with sodium bicarbonate. The ptomaines reduce ferric chlorid, but not iodic acid. With sugar and concentrated sulphuric acid, they give a yellow coloration.

Many extracts from the stomach and liver in toxicological examinations do reduce iodic acid promptly.

Mixtures of the ptomaïns and morphin give absolutely characteristic reactions for morphin with sugar and sulphuric acid, the violet coloration appearing distinctly; and by evaporation on the water-bath with sulphuric acid, addition of hydrochloric acid and decomposition with sodium bicarbonate, the violet coloring appears. Iodic acid is reduced by morphin in the presence of ptomaïns, only when the ptomaïns are present in minute quantity. (This statement is not true of all extracts.)

The other reactions for morphin are not applicable in the presence of ptomaïns.

STRYCHNIN. — The characteristic color-reaction for this alkaloid, with potassium bichromate and sulphuric acid, is not affected by the presence of ptomaïns.¹

BRUCIN. — The nitric acid reaction for brucin is not affected by ptomaïns. On the other hand, the reaction with sulphuric and nitric acids, in which a red coloration is obtained, is scarcely visible in the presence of ptomaïns. The action of mercuric nitrate and heat on brucin, by which a violet coloration is produced, is not destroyed by the presence of ptomaïns.

VERATRIN. — The characteristic coloration of veratrin by concentrated sulphuric acid is not influenced by ptomaïns. The same is true of the cherry-red coloration with concentrated hydrochloric acid. On the contrary, the action of sugar and sulphuric acid on veratrin is without result in the presence of ptomaïns.

ATROPIN. — The deep violet coloration produced by fuming nitric acid, subsequent concentration, and the addition of

¹ In contradiction to this, see page 284.

alcoholic potassium hydrate, is not affected by the presence of ptomaines. On the other hand, the characteristic odor produced by the action of sulphuric acid and heat on atropin is scarcely recognizable when ptomaines are present.

NARCEIN.—The blood-red color produced by concentrated sulphuric acid fails in the presence of ptomaines.

COLCHICIN.—Fuming nitric acid colors the ptomaines reddish-yellow, but the violet coloration of colchicin with nitric acid appears in well-defined form, even in the presence of ptomaines. The other reactions for colchicin are valueless when ptomaines are present.

CODEIN.—The blue coloration of codein with concentrated sulphuric acid holds good when ptomaines are present. The same is true of the reaction with sulphuric acid, heat, and the subsequent addition of nitric acid. Fröhde's reagent fails with codein when mixed with ptomaines, inasmuch as the bluish coloration rapidly passes into a brown.

ACONITIN.—Phosphoric acid and concentrated sulphuric acid are without reaction on the alkaloid when mixed with ptomaines.

PICROTOXIN.—The reducing action of picrotoxin on alkaline copper sulphate solution is seriously affected by the presence of ptomaines. The same is true of other tests for this poison.

DELPHININ.—The reaction of delphinin with sulphuric acid and bromin-water, as well as the one with Fröhde's reagent, is so much influenced by the presence of ptomaines that the alkaloid cannot be recognized.

These results are to be accepted with caution, as it is not reasonable to suppose that all ptomaines will affect the

test for the vegetable alkaloids in the same manner or to the same degree. Moreover, there is no proof that TAMBA worked with pure ptomaïns.

TAMBA has also proposed to separate vegetable from putrefactive alkaloids by adding to ethereal solutions of mixtures an equal volume of a saturated ethereal solution of oxalic acid, and allowing to stand, when the oxalates of the vegetable alkaloids will separate in crystalline form, and the oxalates of the ptomaïns will remain in solution. In other words, the oxalates of the vegetable alkaloids are insoluble in ether, while the oxalates of the putrefactive alkaloids are soluble in ether. But, in contradiction to this, BOCKLISH states that the oxalate of cadaverin is insoluble in ether.

The most important work which the toxicologist is called upon to do at present is to isolate and identify beyond all question the bacterial poisons. This work has become important on account of the frequent occurrence of poisoning from articles of infected food.

CHAPTER XII.

CHEMISTRY OF THE PTOMAÏNS.

THE basic substances described in the following pages are arranged, as far as possible, in the regular natural order. An inspection of the list of these bases will show the remarkable fact of the predominancy of the amine type. Almost two-thirds of the known ptomaïns contain only C, H, and N, and represent simple ammonia substitution-compounds. Of the oxygenated bases, all of those whose constitution is known possess the trimethylamin-molecule as their basic constituent, and it is quite probable that most, if not all, of the remaining ptomaïns will be found to possess the same or a similar basic nucleus.

It will be seen, furthermore, that a very large number of the ptomaïns described possess little or no toxic action, and are, therefore, physiologically inert. It would seem, as BRIEGER has already pointed out, that a certain quantity of oxygen is necessary to the formation of poisonous bases. A free supply of oxygen, on the other hand, invariably yields non-toxic ptomaïns. The poisonous bases begin to appear on about the seventh day of putrefaction, and in turn disappear if this is allowed to go on for a considerable period of time.

METHYLAMIN, CH_3NH_2 .—This is the simplest organic base that is formed in the process of putrefaction. It is ammonia in which one atom of hydrogen has been replaced by the methyl radical. It occurs in herring-brine (TOLLENS, 1866; BOCKLISCH, 1885); in decomposing herring, twelve days in spring (BOCKLISCH); in pike, six days in summer (BOCKLISCH); in haddock, two months at a low temperature (BOCKLISCH); in the fermentation of cholin chlorid (HASE-

BROEK). BRIEGER has shown it to be present in cultures of comma bacillus on beef-broth which were kept for six weeks at 37° – 38° . EHRENBERG reported its possible presence in poisonous sausage, and obtained it by growing a bacillus from this source on intestines (1887). In Brieger's method methylamin is found in both the mercuric chlorid precipitate and filtrate. The mercury double salt is readily soluble in water, and can thus be separated from any accompanying cadaverin or putrescin. Methylamin is an inflammable gas of strong ammoniacal odor, and burning with a yellow flame. It is readily soluble in water, and its solutions give reactions similar to those of ammonia. Its salts are, as a rule, also soluble in both water and alcohol.

The HYDROCHLORID, $\text{CH}_3\text{NH}_2\cdot\text{HCl}$, crystallizes in large deliquescent plates. On being heated with alkali, it gives off the odor of methylamin.

The PLATINOCHLORID, $(\text{CH}_3\text{NH}_2\cdot\text{HCl})_2\text{PtCl}_4$ (Pt = 41.31 per cent.),¹ yields hexagonal plates which usually occur heaped up in several layers. It is soluble in about fifty parts of water at ordinary temperature, and can be readily recrystallized from hot water. It is insoluble in absolute alcohol and in ether.

The AUROCHLORID, $\text{CH}_3\text{NH}_2\cdot\text{HCl}\cdot\text{AuCl}_3 + \text{H}_2\text{O}$, forms prisms, which are readily soluble in water. There is also a readily soluble picrate.

Methylamin does not possess any toxic action, even when given in fairly large doses. This physiological indifference is shared by nearly all the monamins and diamins that have been obtained among the products of putrefaction.

DIMETHYLAMIN, $(\text{CH}_3)_2\text{NH}$, has been found in putrefying gelatin, ten days at 35° (BRIEGER, 1885); in yeast decomposing in covered vessels for four weeks during summer (BRIEGER); in decomposing perch six days in summer

¹ The percentages given in the following pages are calculated from Au = 196.64 (Kritsch), Pt = 194.46 (Seubert), Cl = 35.37, O = 15.96.

(BOCKLISCH); and in herring-brine (BOCKLISCH, 1886). It has been found in poisonous sausage, and in cultures of a bacillus obtained from this source, on liver and intestines (EHRENBERG, 1887). It is also formed together with trimethylamin, when neuridin hydrochlorid is distilled with sodium hydrate (BRIEGER, I., 23). It occurs in the mercuric chlorid precipitate as well as filtrate. From cadaverin it can be separated by platinum chlorid, since cadaverin platinum-chlorid is difficultly soluble in cold water, and recrystallizes from hot water, whereas the dimethylamin double salt remains in the mother-liquor. In like manner it can be separated from neuridin. From cholin it can be isolated by recrystallizing the mercuric chlorid precipitate from hot water.

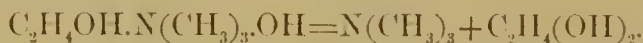
The free base is a gas at ordinary temperature, but can be condensed to a liquid which boils at 8° – 9° . The hydrochloride, $(\text{CH}_3)_2\text{NH}.\text{HCl}$, crystallizes in needles, which deliquesce on exposure to air and are soluble in absolute alcohol (BRIEGER, I., 56). It is insoluble in absolute alcohol (BOCKLISCH), but soluble in chloroform (BEHREND), and can then be separated from methylamin hydrochlorid, which is insoluble in chloroform.

The PLATINOCHLORID, $[(\text{CH}_3)_2\text{NH}.\text{HCl}]_2\text{PtCl}_4$ ($\text{Pt} = 39.00$ per cent.), crystallizes in long needles, which are easily soluble in hot water, less soluble in cold water. Sometimes it forms orange-yellow plates or prisms, or else small needles.

The AUROCHLORID, $(\text{CH}_3)_2\text{NH}.\text{HCl}.\text{AuCl}_3$, forms needles (BOCKLISCH), or large yellow monoclinic plates (HJORTDAHL), which are insoluble in absolute alcohol.

TRIMETHYLAMIN, $\text{C}_3\text{H}_9\text{N} = (\text{CH}_3)_3\text{N}$, has been known for a long time to occur in animal and vegetable tissues. DESSAIGNES showed its presence in leaves of *Chenopodium* (1851), in the blood of calves (1857), and later in human urine. It has been obtained from ergot (*Secale cornutum*) by WALZ (1852) and BRIEGER (1886); from herring-brine by WERTHEIM, WINKLES, TOLLENS, and BOCKLISCH. In these substances, with the exception of herring-brine, it probably

does not exist pre-formed, but is rather a product of the method employed for its isolation. In fact, BRIEGER has shown that it does not exist in ergot, but is formed at the expense of the cholin present, which, on distillation with potash, decomposes and yields trimethylamin and glyeol. Thus:



It is also formed when betain and neuridin are distilled with potash. It may have a similar origin in most of the other cases, since cholin is now known to be widely disseminated in plants and animals, either as such or as a constituent of the more complex lecithin. Trimethylamin has been found in the putrefaction of yeast (HESSE, 1857; MÜLLER, 1858); in cheese after six weeks in midsummer (BRIEGER); in human liver and spleen after from two to seven days (BRIEGER); in perch after six days in midsummer (BOCKLISCH); in mussel (*Mytilus edulis*) after sixteen days (BRIEGER); in putrefying brains after from one to two months, and in fresh brains (GUARRESCHI and Mosso); in cultures of the streptococcus pyogenes on beef-broth, bouillon, meat-extract, and blood-serum; from cultures of the comma-bacillus (BRIEGER), and from cultures of proteus vulgaris (CARBONE). It has also been found in cod-liver oil. EHRENBERG (1887) reports its presence in considerable quantity in poisonous sausage, and in cultures of a bacillus, isolated from this, grown on liver, intestines, and meat-bouillon. STADTHAGEN has found it in normal urine; KULNEFF in the feces of a case of gastropse.

Trimethylamin is found in both the mercuric chlorid precipitate and filtrate. It remains in the mother-liquor from which cadaverin, neuridin, and dimethylamin platinochlorids have crystallized. If an aqueous solution of mercuric chlorid is used as the precipitant, the trimethylamin will be found almost entirely in the filtrate, from which it can be obtained after removal of the mercury by evaporating the filtrate to dryness, extracting with alcohol, and treating the solution thus obtained with alcoholic platinum chlorid.

The free base is a liquid possessing a strong, fish-like odor. Its boiling-point is 9.3° . It is strongly alkaline in reaction and freely soluble in water.

The HYDROCHLORID, $(\text{CH}_3)_3\text{N}.\text{HCl}$, is deliquescent and freely soluble in water and alcohol. Heated to 285° it decomposes. With alkalis it gives off the odor of the free base.

The PLATINOCHLORID, $[(\text{CH}_3)_3\text{N}.\text{HCl}]_2\text{PtCl}_4$ ($\text{Pt} = 36.92$ per cent.), is soluble in hot water, from which, on cooling, it recrystallizes in orange-red octahedra or needles, which do not lose water when heated at 100° – 110° (BOCKLICH).

The AUROCHLORID, $(\text{CH}_3)_3\text{N}.\text{HCl}.\text{AuCl}_3$ ($\text{Au} = 49.39$ per cent.), is easily soluble, and hence can be separated from cholin aurochlorid, which is difficultly soluble. Similarly this base can be separated from ammonia by the use of gold chlorid.

Trimethylamin is not a strong poison, since very large doses of it must be given in order to bring out any physiological disturbances.

ETHYLAMIN, $\text{C}_2\text{H}_5.\text{NH}_2$, is formed in putrefying yeast (HESSE, 1857); in wheat flour (SULLIVAN, 1858); and also in the distillation of beat-sugar residues.

It is a strongly ammoniacal liquid boiling at 18.7° and is miscible with water in every proportion. Like the other amines, it is combustible. It possesses strong basic properties, and is capable of expelling ammonia from its salts in a manner analogous to the action of the fixed alkalis.

The HYDROCHLORID, $\text{C}_2\text{H}_5.\text{NH}_2.\text{HCl}$, forms deliquescent plates, which melt at 76° – 80° . It is readily soluble in water and alcohol.

The PLATINOCHLORID, $(\text{C}_2\text{H}_5.\text{NH}_2.\text{HCl})_2\text{PtCl}_4$, forms orange-yellow rhombohedra (WELTZIEN), or hexagonal-rhomboidal crystals (TORSOË).

The AUROCHLORID, $\text{C}_2\text{H}_5.\text{NH}_2.\text{HCl}.\text{AuCl}_3$, forms gold-yellow monoclinic prisms, readily soluble in water.

With picric acid it forms short brown prisms, not very soluble in water.

DIETHYLAMIN, $C_4H_{11}N = (C_2H_5)_2NH$, has been obtained by BOCKLISCH from pike which were allowed to putrefy for six days in summer; and by growing a bacillus obtained from poisonous sausage on intestines and on meat-bouillon (EHREXBERG, 1887).

It is an inflammable liquid which boils at 57.5° , possesses strong basic properties, and is soluble in water.

The HYDROCHLORID, $(C_2H_5)_2NH.HCl$, crystallizes in needles (BOCKLISCH); in long needles and prisms from absolute alcohol; in plates from ether-alcohol. These are not deliquescent and are easily soluble in water and in chloroform; rather difficultly in absolute alcohol. Heated with sodium hydrate it gives off alkaline vapors. From an alcoholic solution it is precipitated by addition of alcoholic mercuric chlorid. The mercury double salt is difficultly soluble in hot water, from which it recrystallizes on cooling.

The PLATINOCHLORID, $[(C_2H_5)_2NH.HCl]_2PtCl_4$, crystallizes in orange-yellow monoclinic crystals, which are easily soluble in water.

The AUROCHLORID, $(C_2H_5)_2NH.HCl.AuCl_3$ ($Au = 47.71$ per cent.), forms trimetric crystals (TOPSOË), which are difficultly soluble (BOCKLISCH). It melts at about 165° .

With picric acid it forms an easily soluble picrate (LEA).

TRIETHYLAMIN, $C_6H_{15}N = (C_2H_5)_3N$, was obtained by BRIEGER (1885) from haddock which were exposed for five days in an open vessel during summer. He obtained it by distilling with potash, after removal of platinum by hydrogen sulphid, the mother-liquor from which neuridin, the base $C_2H_5N_2$, muscarin, and gadinin had successively crystallized (see Gadinin). It has also been found by BOCKLISCH (1886) in putrid pike, and by EHREXBERG (1887). The latter obtained it from cultures of a bacillus, found in poisonous sausage, and grown on meat-bouillon.

The free base is oily in character and possesses an ammoniacal odor. It is but slightly soluble in water, and boils at $89^\circ-89.5^\circ$.

The PLATINOCHLORID, $[(C_2H_5)_3N.HCl]_2PtCl_4$ (Pt=31.84 per cent.), crystallizes in needles which are readily soluble in water.

With mercuric chlorid the aqueous solution gives no precipitate.

With picric acid it yields yellow needles which are but slightly soluble in cold water.

PROPYLAMIN, $C_3H_7.NH_2$, is isomeric with trimethylamin, and can therefore be easily confounded with that base. There are two propylamins possible represented by the formulæ $CH_3.CH_2.CH_2.NH_2$ and $(CH_3)_2.CH.NH_2$. The former, or the normal compound, boils at $47^\circ-48^\circ$, whilst the latter, or iso-propylamin, boils at 31.5° . Both are liquids possessing an ammoniacal, fish-like odor. They form crystalline salts; the hydrochlorids melt respectively at $155^\circ-158^\circ$ and at 139.5° .

Iso-propylamin(?) has been found among the distillation-products of the vinasse of beet-root molasses. Propylamin has been obtained by BRIEGER (1887) from cultures of the bacteria of human feces on gelatin. SCHWANERT has isolated from the organs of a cadaver a basic substance which was said to possess an odor similar to propylamin.

BUTYLAMIN, $C_4H_{11}N$, was obtained by GAUTIER and MOURGUES (1888) in cod-liver oil. It forms a colorless, mobile, alkaline liquid, the boiling point of which they found to be 86° at 760 mm. It absorbs carbonic acid from the air and readily forms salts. The platinochlorid forms golden-yellow plates which are quite soluble.

In animals it produces an increase in the function of the skin and kidneys, and in large doses fatigue, stupor, and vomiting.

ISO-AMYLAMIN, $C_5H_{13}N=(CH_3)_2.CH.CH_2.CH_2.NH_2$, has been obtained by LIMPRICHT in the distillation of horn with potash; it also occurs in the putrefaction of yeast (MÜLLER, HESSE,

1857); and in cod-liver oil (GAUTIER and MOURGUES) 1888), where it constitutes nearly one-third of the bases present.

It is a colorless, strongly alkaline liquid, possessing an odor which is not disagreeable. At the ordinary pressure it boils at 97° – 98° .

The hydrochlorid forms deliquescent crystals, which have a bitter, disagreeable taste. The platinochlorid crystallizes in golden-yellow slender plates, which are very soluble in boiling water. The base is, according to GAUTIER and MOURGUES, identical with that obtained by treating iso-amylearbimide with potash.

It is a very active poison, producing rigor, convulsions, and death. Four milligrams produce death in a greenfinch in three minutes.

CAPROYLAMIN (HEXYLAMIN), $C_6H_{15}N$, has been found to occur by HESSE (1857) in the putrefaction of yeast. HAGER isolated from some putrid material what he thought to be a mixture of amylamin and caproylamin, and named it septiem.

Hexylamin was found, in small quantity, in cod-liver oil, by GAUTIER and MOURGUES, and according to these authors it resembles amylamin in its action, but is less toxic.

TETANOTOXIN, $C_5H_{11}N(?)$, was obtained by BRIEGER (1886) as one of the products of the growth of the tetanus-microbe on beef-broth or on brain-broth. It has also been obtained by KITASATO and WEYL (1890) from pure cultures of the tetanus-bacillus, kept eight days at 36° . For its isolation see Tetanin, and *Ber.* **19**, 3120. It is tetanizing in its action, produces first tremor, then paralysis and violent convulsions. It forms an easily soluble gold double salt which melts at 130° . The platinochlorid is difficultly soluble, and decomposes at 240° . The hydrochlorid is crystalline, and is readily soluble in alcohol and in water. It melts at about 205° . From warm alcohol it crystallizes in flat, pointed plates.

SPASMOTOXIN, a base of as yet unknown composition, produces in animals violent clonic and tonic convulsions. It was obtained by BRIEGER (1887) from cultures of the tetanus-germ on beef-broth.

Another *toxin* was obtained by BRIEGER (1887) in cultures of the tetanus-microbe which produced complete tetanus, salivation, and tear-secretion. In its composition it is probably a diamine. The platinochlorid forms plates which begin to decompose at 240° . The hydrochlorid is very deliquescent. Gold chlorid and picric acid form very soluble compounds. Besides these three bases he isolated another toxic substance, tetanin, and a base (see under Tetanin).

DIHYDROLUTIDIN, $C_7H_{11}N$, was found in cod-liver oil by GAUTIER and MOURGUES (1888). It is the first known hydrolutidin. It is a colorless, somewhat oily, very alkaline and caustic liquid, the odor of which is sharp, but somewhat agreeable when dilute. It absorbs carbonic acid from the air, darkens and thickens; is feebly soluble in water, and boils at 199° at 760 mm. pressure. The salts are bitter to the taste.

The hydrochlorid crystallizes in a confused mass of needles or in plates. The nitrate reduces silver nitrate—a property of all hypopyridin-bases (HOFMANN). The sulphate forms fine stellate deliquescent needles.

The platinochlorid is readily precipitated from concentrated solutions as a canary-yellow precipitate. From warm solutions it crystallizes in lozenge-shaped plates which are often imbricated. On boiling with water it loses hydrochloric acid and forms $(C_7H_{11}NCl)_2PtCl_2$, which possesses a lighter color, is more soluble than the normal salt, and crystallizes confusedly.

The anrochlorid crystallizes in needles which form fan or lozenge-shaped masses. It is scarcely altered even in hot water.

The IODOMETHYLATE, $C_2H_{11}N.CH_3I$, is obtained by mixing, in the cold, the base and methyl iodid. The colorless compound thus obtained is soluble in water and in alcohol, and

possesses a disagreeable, somewhat nauseating odor. Treated with potash it yields a colorless, aromatic, very alkaline oil.

The base on oxidation with boiling potassium permanganate yields an acid, $C_7H_7NO_2$, and from this fact the discoverers conclude that the base is a dihydro-dimethylpyridin, $C_5H_4(CH_3)_2NH$.

Physiological Action.—It is moderately poisonous. In small doses it diminishes the general sensibility; in larger doses it produces trembling, especially of the head; profound depression alternating with periods of extreme excitement; paralysis of the posterior limbs, and death.

A BASE, $C_8H_{11}N$, isomeric, but not identical, with aldehyde-collidin, was obtained by NENCKI as early as 1876, by allowing a mixture of 200 grams of pancreas and 600 grams of gelatin in ten litres of water to putrefy for five days at 40° . The method used by NENCKI for its isolation is as follows: The fluid mass was distilled with sulphuric acid, to drive off the volatile acids, then rendered alkaline with barium hydrate, and again distilled. The distillate was received in dilute hydrochloric acid, and on evaporation gave a crystalline residue of ammonium chlorid, and of a salt which formed in long rhombic plates. The latter were separated from the ammonium salt by absolute alcohol. The free base was obtained from the salt by treating it with sodium hydrate, and extracting the solution with ether.

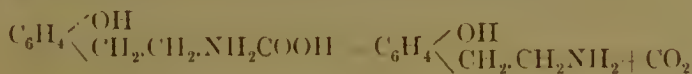
This compound, as already stated, is isomeric with collidin, and also with O. DE CONINCK's base, with which it is possibly identical. The latter, however, will be described separately.

The free base is oily in character, and possesses a peculiar, not unpleasant odor. It readily absorbs carbonic acid gas from the air, forming after a time a lamellar, crystalline mass of the carbonate. The salt of this base on heating gives off an oil which burns with a smoky flame, and possesses an odor similar to that of xylol or cumol. NENCKI was therefore at first of the opinion that the ptomain was an aromatic base, probably an isophenyl-ethylamin of the following composition:

$\text{C}_6\text{H}_5-\text{CH} \begin{smallmatrix} \diagup \text{CH}_3 \\ \diagdown \text{NH}_2 \end{smallmatrix}$. He supposed that it was formed from the putrefaction of tyrosin, according to the following equation:



We know that tyrosin does split up, on being heated to 270° , into carbonic acid and oxyphenyl-ethylamin, thus:



In 1883 ERLÉNMEYER and LIPP observed that phenyl- α -amido-propionic acid (phenyl-alanin), on dry distillation, decomposed with the formation, among other products, of a base having the composition $\text{C}_8\text{H}_{11}\text{N}$. This base was found to be identical with phenyl-ethylamin, $\text{C}_6\text{H}_5.\text{CH}_2.\text{CH}_2.\text{NH}_2$, and in its properties and composition it resembles NENCKI's base. Recently (1889), NENCKI has taken up a similar view in regard to the nature of this base, and now regards it as possessing the formula just given—that it is phenyl-ethylamin. He regards phenyl amido-propionic acid—one of the three aromatic nuclei contained in the albumin-molecule—as the source of this base. From the fact that phenyl α -amido propionic acid is a well-known putrefactive product, it would seem that NENCKI's base may arise either from the putrefactive decomposition of that acid, or from the splitting up of the acid as a consequence of the method employed in isolating the base. The latter would seem to be the most probable explanation of the genesis of this base, inasmuch as BRIEGER, by using his method for the isolation of ptomains, has not been able to obtain it from putrid gelatin.

The PLATINOCHLORID, $(\text{C}_8\text{H}_{11}\text{N}.\text{HCl})_2\text{PtCl}_4$ (Pt=29.89 per cent.), is readily soluble in hot, and but slightly soluble in cold water, and can be, therefore, recrystallized from water. It forms beautiful flat needles. On dry heating it gives off an oil which possesses an odor resembling very much that of xylol or annol, and burns with a smoky flame. This distin-

guishes NENCKI'S base from collidin, since the platinochlorid of the latter does not show this behavior.

NENCKI also obtained from putrid gelatin, under certain ill-defined conditions, especially when no glycocoll was present, a basic product which gave, with sulphuric acid, large lamellar crystals. The free base forms a thick colorless syrup, possessing a nauseous, bitter taste. It did not become crystalline even after standing some time. Unlike the base $C_8H_{11}N$, it is not volatile, and is, therefore, obtained on evaporation of the acidulated solution after previous removal of the volatile bases by distillation with baryta.

A BASE, $C_8H_{11}N$, isomer of collidin and of the preceding base, with which it is possibly identical, was obtained by O. DE CONINCK (1888) in the later stages of putrefaction of sea-polyps (*poules marins*). It forms a yellowish, rather mobile liquid, possessing a strong benumbing (*vireuse*) odor, and is but slightly soluble in water. It is soluble in methyl and ethyl alcohol, ether and acetone. Its density is 0.9865. When dried over potash it boils at 202° without undergoing decomposition. On exposure to the air it becomes brown, hydrates rapidly, and the boiling point is then lowered. It has not been noticed to absorb carbonic acid from the air. It resembles some of the bases obtained from Dippel's oil. The salts are in general less stable than those of the pyridin-bases, and in this respect it approaches the dihydro-pyridin-bases.

The HYDROCHLORID, $C_8H_{11}N.HCl$, forms white or slightly yellowish radiate masses which are deliquescent and very soluble in water. The hydrobromid, $C_8H_{11}N.HBr$, resembles it, but is less deliquescent and a trifle less soluble in cold water.

The PLATINOCHLORID, $(C_8H_{11}N.HCl)_2PtCl_4$, is a dark orange-colored powder, which is insoluble, or almost so, in cold water, and is a rather stable compound. Boiling water and water at 80° decompose it into hydrochloric acid and $(C_8H_{11}NCl)_2PtCl_2$, which is a light-brown powder, insoluble in cold, scarcely so in hot water.

The AUROCHLORID, $C_8H_{11}N.HCl.AuCl_3$, forms a light-

yellow precipitate. It is quite stable in cold, but very unstable in hot or even warm water. It cannot be modified by withdrawal of hydrochloric acid.

It forms two compounds with mercuric chloride. $(C_8H_{11}N \cdot HCl)_2HgCl_2$ crystallizes in small white needles, which are slightly soluble in water and in dilute alcohol, insoluble in absolute alcohol, and on exposure to moist air undergo change. The second compound, $2(C_8H_{11}N \cdot HCl) \cdot 3HgCl_2$, is obtained by adding an excess of concentrated mercuric chlorid to a concentrated solution of the hydrochlorid. It forms slightly yellow, somewhat longer needles which are insoluble in the principal solvents, and are likewise changed by atmospheric humidity.

The IODOMETHYLATE, $C_8H_{11}N \cdot CH_3I$, is formed by mixing solutions of the base and methyl iodid in absolute ether. It is deposited as a network of fine white needles, which are but slowly altered in the air, and are soluble in absolute alcohol. This solution on the addition of a little potash assumes a dark-red color, which is heightened by the addition of a little hydrochloric or acetic acid, and destroyed by ammonia without any resultant fluorescence. Warmed with excess of moist solid potash it becomes garnet-red in color and gives off an odor resembling that of the dihydropyridins. It thus behaves the same as the pyridin iodomethylates.

On oxidation with potassium permanganate it yields an acid which melts at 229° – 230° , and begins to sublime at 150° . It presents all the characteristics of nicotinic acid, $C_6H_5NO_2$, which is formed as the result of oxidation of nicotine. With hydrochloric acid it forms the compound $C_6H_5NO_2 \cdot HCl$. With copper acetate it forms a salt; this, distilled with lime, yields a substance which on boiling with platinum chlorid and water forms the compound $(C_5H_5NCl)_2 \cdot PtCl_2$. This same substance forms an iodomethylate, which in alcoholic solution gives, on addition of potash, the characteristic reaction of pyridin-bases.

The base $C_8H_{11}N$, therefore, yields pyridin and nicotinic acid.

A BASE, $C_8H_{13}N$, was obtained by GAUTIER and ETARD (1881) from the chloroformic extracts (see method, page 270) from putrefying mackerel, as well as from the decomposing flesh of the horse and ox. It is regarded by these authors as a constant and definite product of the bacterial fermentation of albuminoid substances, but this view is hardly justifiable, inasmuch as the base has not been found by other investigators. It is accompanied by the base $C_{17}H_{38}N_4$ (page 351). NENCKI (1882) asserted the identity of this base with the one which he had isolated in 1876, and to which he had ascribed the formula $C_8H_{11}N$. On the other hand, GAUTIER and ETARD consider their base to be identical with the hydrocollidin obtained by CAHOUS and ETARD by the action of selenium on nicotine.

The free base is an alkaline, almost colorless, oily liquid, possessing a penetrating odor resembling that of syringa. It is volatile without decomposition, and boils at about 205° , while hydrocollidin boils at 210° . Its density at zero is 1.0296. When exposed to the air it oxidizes slowly, becomes brown and viscous, and at the same time absorbs carbonic acid. It differs from a collidin in possessing a strong reducing action, since both the gold and platinum double salts become reduced on heating, and even in the cold.

The HYDROCHLORID, $C_8H_{13}N.HCl$, is very soluble in water and in alcohol, and usually forms fine needles resembling snow-crystals. It is neutral in reaction and possesses a bitter taste. In the presence of an excess of acid it reddens and resinifies.

The PLATINOCHLORIDE, $(C_8H_{13}N.HCl)_2PtCl_4$ ($Pt=29.7$ per cent.), is of a light-yellow, flesh color, crystalline, and but slightly soluble. It dissolves on warming, and recrystallizes in bent needles.

The AUROCHLORID is rather soluble, and becomes slowly reduced in the cold; rapidly on warming.

Physiological Action.—This isomer of hydrocollidin is strongly poisonous. Even so small a dose as 0.0017 gram of the hydrochlorid produced, when injected under the skin of a bird, marked unsteadiness of gait, followed by paralysis

of the extremities, and finally death. The pupils are normal and the heart stops in diastole. Larger doses (0.007 gram) cause at first vomiting and staggering, which soon give way to a condition of exaltation. Toward the end tetanic convulsions set in, followed by almost complete paralysis.

A BASE, $C_9H_{13}N$, isomeric with parvolin, has been extracted by GAUTIER and ETARD (1881) from decomposing mackerel and horseflesh. The method employed by these chemists for its isolation is given on page 270. The identity of this base with the synthetic parvolin, obtained by WAAGE by heating ammonia with propionic aldehyde in a sealed tube at 200° , cannot be considered to be definitely settled, although an apparent identity exists in regard to their boiling-points. Thus, the synthetic parvolin boils at 193° – 196° , while GAUTIER and ETARD assign to their base a boiling-point a little below 200° . Further investigation is necessary to decide upon the question of the identity of this base with parvolin, or of the ptomain $C_8H_{13}N$ with hydrocollidin.

The free base is an oily, amber-colored liquid, possessing the odor of hawthorn-blossoms. It is slightly soluble in water; very soluble in alcohol, in ether, and in chloroform. Its boiling-point, as stated above, is a trifle below 200° . Like the bases $C_8H_{13}N$ and $C_{10}H_{15}N$ it becomes brown and soon resinifies on exposure to air.

The PLATINOCHLORID, $(C_9H_{13}N.HCl)_2PtCl_4$ (Pt = 28.65 per cent.), is slightly soluble, crystalline, and flesh colored; exposed to the air it soon becomes pink.

The AUROCHLORID is quite soluble.

A BASE, $C_{10}H_{15}N$, was isolated by GUARESCHI and Mosso (1883) from ox-blood fibrin which had been allowed to putrefy for five months. In 1887 it was re-obtained from putrid fibrin by GUARESCHI, who this time ascribed to it the formula $H^0(C_{10}N$. In 1886 OECHSNER DE CONINCK found it among the basic products formed in the putrefaction of the jelly-fish (*pouupes marins*, HUGOUENQ, page 21). The method used

for its extraction was that of GAUTIER and ETARD (see page 270). It forms a brownish oil of strong alkaline reaction, which soon resinifies. It possesses an unpleasant, weak pyridin- or coniin-odor, and is but slightly soluble in water; soluble in ether and in chloroform.

In regard to the constitution of this ptomain we know nothing, but from its physical characters it would seem to possess a pyridin nucleus. It is isomeric with corindin, a homologue of parvolin and collidin, which has been obtained from coal-tar.

For the behavior of the hydrochlorid to alkaloidal reagents, see Table I.

The HYDROCHLORID, $C_{10}H_{15}N.HCl$, crystallizes in colorless cholesterin-like plates which are somewhat deliquescent.

The PLATINOCHLORID, $(C_{10}H_{15}N.HCl)_2PtCl_4$ ($Pt = 27.52$ per cent.), forms a light flesh-colored, crystalline precipitate, and is insoluble in water, alcohol, and ether. It does not resinify, and is stable at 100° .

Physiological Action.—This ptomain resembles curara, although it is by no means so strong. 0.012 gram of the free base produced in a frog dilatation of the pupil and slowing of the respiration. The nostrils were motionless, and within five hours complete paralysis of the muscles took place. The reflex excitability gradually diminished until it finally disappeared. An orange-blossom odor was observed about the frogs which were poisoned by this ptomain. The same amount of ptomain injected into a greenfinch produced vomiting, and a condition of weakness and decreased sensibility, followed soon, however, by recovery. A rat was not affected by 0.020 gram of the free base. The hydrochlorid acts much more energetically.

A BASE, $C_{10}H_{15}N$, was isolated by O. DE CONINCK, in 1886 (HUGOUNENQ, page 21, *C. Rendus*, 1888), from sea-polyps in an advanced stage of putrefaction, together with the base $C_8H_{11}N$. The method employed for its extraction was that of GAUTIER and ETARD (see page 270). It forms a slightly

yellow, viscous liquid, and possesses a pleasant odor resembling that of blooming broom. Its density is about 1.18. It boils at about 230° (uncorrected), with initial decomposition. In water it is but slightly soluble, readily so in ether, alcohol, acetone, and ligroin. It is rapidly oxidized by the air, becomes brown, and resinifies, but does not absorb carbonic acid.

The HYDROCHLORID, $C_{10}H_{15}N.HCl$, forms fine yellowish, very deliquescent needles, which in the presence of a trace of air are at once colored red; if more air is present, the red changes to a brown, and in the open air a resin is formed the same as from the free base. It is very easily soluble.

The HYDROBROMID, $C_{10}H_{15}N.HBr$, crystallizes in a network of fine deliquescent needles, which become likewise red on exposure to air. It is very soluble in water; less so in strong alcohol, and almost insoluble in ether.

The PLATINOCHLORIDE, $(C_{10}H_{15}N.HCl)_2PtCl_4$, forms a dark-red powder, which is insoluble in cold water; very soluble in warm water. It can be kept in dry air; in moist air it loses hydrochloric acid and becomes partially oxidized. Boiling water decomposes it. $(C_{10}H_{15}N.Cl)_2PtCl_2$ forms clear-brown plates, which are stable in moist air, and melt at 206° . It is insoluble in cold water, soluble in boiling water, but decomposes. In recrystallizing, warm previously boiled water should be used.

The AUROCHLORID, $C_{10}H_{15}N.HCl.AuCl_3$, occurs as a light-yellow precipitate; insoluble in cold water, soluble in warm water. It is decomposed by boiling water; is stable when kept in a moist atmosphere.

The IODOMETHYLATE, $C_{10}H_{15}N.CH_2I$, in warm alcoholic solution yields, on the addition of strong potash, a bright-red color, which soon becomes brown, and in about an hour the solution shows a greenish-blue fluorescence. This rapidity of change is due to the extreme oxidizability of the ptomain.

On careful oxidation with potassium permanganate at ordinary temperature it yields a solid acid, having a melting-point of 228° – 229° , the same as that of the pyridin carbonic acid of HUBER and LAIDLIN, obtained by the oxidation of

nicotin. The solubility in cold and in warm water and in absolute alcohol is the same as that of nicotinic acid. It begins to sublime at 150° as pearly spangles. The formula is $C_6H_5NO_2$. In distillation with lime pyridin forms. It is, therefore, identical with nicotinic acid, an oxidation product of nicotin and other volatile alkaloids.

O. DE CONINCK considers this base, as well as $C_8H_{11}N$, as belonging to the pyridin, and not to the hydropyridin series.

A BASE, $C_{10}H_{17}N$, was described by GRIFFITHS (1890) as derived from cultures on pepton-agar of the bacterium *allii*, a germ obtained from putrid onions. The base (hydrochlorid?) forms colorless, prismatic, microscopic, very deliquescent needles, which are soluble in warm water, alcohol, ether, and chloroform. It gives a hawthorn-like odor, especially when warmed. With phosphomolybdic acid it yields a white; with iodine in potassium iodide and with tannic acid a chestnut-colored precipitate. NESSLER'S solution produces a yellow chestnut-colored precipitate. Picric acid throws down a yellow slightly soluble deposit. The platinochloride, $(C_{10}H_{17}N.HCl)_2PtCl_6$, is yellow, crystalline, and difficultly soluble in cold water and in alcohol; soluble in warm water. Gold chlorid produces a thick yellow precipitate soluble in water. Dilute sulphuric acid produces a *violet-red* color. The base is apparently a hydrocoridin.

During the past five years GRIFFITHS has described, together with a variety of other products in physiological chemistry, the basic compounds which are given in the subjoined table. The method employed in the isolation of all but few of these compounds is that of LUFF (see next chapter under Urine).

Bacillus allii,		$C_{10}H_{17}N$. . .	1890.
Scarlet-fever urine,	Scarlatinin,	$C_5H_9NO_4$. . .	1891.
Diphtheria "	Diphtherin,	$C_{14}H_{17}N_2O_6$. . .	"
Parotitis "	Propyl-glycoeyamin,	$C_6H_{13}N_3O_2$. . .	"
Glanders "		$C_{15}H_{16}N_2O_6$. . .	1892.
Pneumonia "		$C_{20}H_{26}N_2O_3$. . .	"
Measles "	Glycoeyamidin,	$C_3H_5N_3O$. . .	"

Whooping-cough urine,		$C_5H_{10}NO_2$. . .	1892.
Erysipelas	Erysipelin,	$C_{11}H_{13}NO_3$. . .	"
Puerperal-fever	Puerperalin,	$C_{22}H_{19}NO_2$. . .	"
Epilepsy	"	$C_{12}H_{16}N_5O_7$. . .	"
Micrococcus tetragenus,		$C_5H_6NO_2$. . .	"
Bacillus phylatilis,		$C_9H_{21}N_2O_5$. . .	"
Eczema urine,	Eezemiu,	$C_7H_{15}NO$. . .	1893.
Influenza	"	$C_9H_9NO_4$. . .	"
Putrid sardines,	Sardinin,	$C_{11}H_{11}NO_2$. . .	"
Cancer urine,	Cancerin,	$C_8H_5NO_5$. . .	1894.
Pleurisy	Pleuricin,	$C_5H_5O_2$. . .	"
Angina-pectoris urine,		$C_{10}H_9NO_4$. . .	1895.

It is very suspicious to find such a long array of products, isolated apparently with the greatest ease by a most simple method. This is all the more remarkable in view of the fact that other skilled workers have utterly failed in some of these cases to isolate basic substances. Thus cultures of the Loeffler-bacillus of diphtheria have been shown by BRIEGER and FRAENKEL and others not to contain ptomains, and yet GRIFFITHS claims to isolate a base from the urine in the disease, and also from the pure cultures of the bacillus. The base is apparently so abundant that in 1893 he was able to sacrifice 1.5 g.(!) in order to show that a disinfectant "izal" destroyed its poisonous properties(!). Again, from four gallons of scarlet fever urine LUFF succeeded in obtaining some crystals of a base insufficient, however, for analysis. GRIFFITHS, however, not only isolated it in sufficient quantity, but had 2.5 g. of it to spare for testing the power of "izal." Further than that, he succeeded in isolating scarlatinin from pure cultures of the micrococcus scarlatinae(!). We have yet to learn that scarlet fever is due to a micrococcus.

Equally interesting facts appear in connection with the base of glanders, which was isolated from the urine and also from pure cultures of the glanders-bacillus. NENCKI, however, has had 10 litres of a bouillon-culture of the glanders-bacillus examined according to GRIFFITHS's method, and failed to obtain a weighable quantity of a ptomain (MALY's *Jahresbericht*, 1894, 24, 601). The action of this basic product of glanders is remarkable, producing "an abscess at the point of inoculation, nodules in the lungs and spleen, and metastatic abscesses in various organs."

The description of these bases is so brief and unsatisfactory that the description of one applies almost equally well to all the others. Thus, the bases are all white, crystalline, and soluble in water, imparting an alkaline reaction in all but two or three cases. All but four or five are characterized as poisonous, but the dose employed is never given; the method of administration is mentioned but twice, and the kind of animal employed but six times. All, or nearly all, form crystalline hydrochlorids, anrochlorids, and platinochlorids. Reactions are usually given with only three or four reagents. It will be noticed that one of these products has no nitrogen and yet is mentioned as a ptomain (!); further, that the formula of five of these compounds is not in accord with the law of even numbers. Considering the amounts of the bases available for "izal" experiments, it is proper to expect accurate, exhaustive, thorough work. Chemical science is not advanced by coining names or establishing formulæ.

A BASE, $C_{32}H_{31}N$, was obtained by DELÉZENIER (1889) and is said to be the alkaloid isolated in 1879 by BROUARDEL, which in its chemical and physiological properties was described as similar to veratrin. It forms an almost colorless oily fluid, which possesses a hawthorn-like odor. It is very readily oxidizable and yields the veratrin-like reactions only in the presence of air. It is soluble in alcohol, ether, toluene, and benzene; and forms well-defined salts which are very deliquescent. It appears to be an amin, and in its composition differs from cevadin by $9H_2O$. Nothing is stated in regard to its source or method of preparation. The analytical results given—C=89.41, H=7.3, N=3.03—correspond more to the formula $C_{34}H_{33}N$.

ETHYLEDIENEDIAMIN(?), $C_2H_8N_2$.—This base was considered at first by BRIEGER to be identical with ethylenediamin, but subsequent comparison showed this to be an error. Thus, the former is poisonous and does not form a gold salt, while the latter is not poisonous and does form a rather difficultly soluble gold salt. Again, ethylenediamin forms a platinochlorid

which is almost insoluble in hot water, whereas the platinum double salt of the ptomain is much more easily soluble. BRIEGER is, therefore, inclined to think that it is identical with ethylenediamin, $\text{CH}_3\cdot\text{CH}(\text{NH}_2)_2$, rather than with ethylenediamin, which has the structure $\text{CH}_2\cdot\text{NH}_2\cdot\text{CH}_2\cdot\text{NH}_2$. This ptomain was obtained by BRIEGER, 1885 (I., 44), from decomposing haddock (see Gadinin). KULNEFF has probably met with this base in the liquids of the stomach in gastrectasis. CARBONE has reported it in cultures of the proteus vulgaris with gadinin, trimethylamin, and cholin.

The free base can be obtained, without decomposition, on distilling the hydrochloride with sodium hydrate.

The HYDROCHLORIDE, $\text{C}_2\text{H}_8\text{N}_2\cdot 2\text{HCl}$, crystallizes in long glistening needles which are readily soluble in water, insoluble in absolute alcohol. It gives no combination with gold chlorid. For its behavior to alkaloidal reagents see Table I.

The PLATINOCHLORID, $\text{C}_2\text{H}_8\text{N}_2\cdot 2\text{HCl}\cdot\text{PtCl}_4$ ($\text{Pt}=41.49$ per cent.), forms small yellow plates which are moderately difficultly soluble in water. It can be readily recrystallized from hot water.

Physiological Action.—Frogs seem to be less susceptible to the action of this poison than mice or guinea-pigs. In the latter, it produces a short time after injection an abundant periodic flow of secretion from the nose, mouth, and eyes. The pupils dilate and the eyeballs project. Violent dyspnoea then comes on and predominates until the death of the animal, which does not take place for twenty-four hours or more. The heart is stopped in diastole.

TRIMETHYLENEDIAMIN(?), $\text{C}_3\text{H}_{10}\text{N}_2$ (?), is a toxic base isolated by BRIEGER (1887) from cultures of the comma bacillus on beef-broth. It may be stated here that from the same source, cholera cultures, KUNZ (1888) obtained a base which he considered to be identical with spermin or ethylenimin (see next chapter). It is present, however, in exceedingly minute quantity, and occurs in the mercuric chlorid precipitate, from which it is obtained by the following method:

The precipitate is decomposed by hydrogen sulphide, the filtrate evaporated to dryness, and the residue taken up with absolute alcohol and precipitated by an alcoholic solution of sodium picrate. The precipitate thus obtained consists of the picrates of cadaverin, creatinin, and of this new base. It is boiled with absolute alcohol to remove the insoluble cadaverin picrate; the filtrate is evaporated to expel the alcohol, and the bases then converted into the platinum double salts, whereby the easily soluble creatinin platinochlorid can be separated from the corresponding less soluble compound of the new base.

Owing to the small quantity of this substance present, a complete study of its properties has not as yet been made. It gives difficultly soluble precipitates with gold chlorid and with platinum chlorid; the compound with the latter crystallizes in long needles. With picric acid it gives a precipitate consisting of felted needles, which resemble creatinin picrate; they melt at 198° . Phosphomolybdic acid yields a precipitate crystallizing in plates, while potassium-bismuth iodid gives dark-colored fine needles. From its physiological action it seems to be identical with the basic substance isolated from choleraic bodies by different observers. It causes violent convulsions and muscle-tremor.

Besides trimethylenediamin another toxin was obtained by BRIEGER from cholera cultures, but in quantity insufficient for analysis. It was obtained from the mercuric chlorid filtrate after elimination of methylamin, trimethylamin, and traces of cholin and creatinin, as an insoluble platinum double salt. Subcutaneous injection of this base into mice produced a paralysis-like lethargic condition, slowing of respiration and heart's action, lowering of temperature, and, finally, death in twelve or twenty-four hours. In some cases bloody stools were passed.

PUTRESCIN, $C_4H_{12}N_2$, is a diamin which almost invariably occurs together with cadaverin, with which it is apparently closely related. This base was also discovered by BRIEGER

in 1885 (II., 42), who obtained it from putrefying human internal organs (for four months at a low temperature without access of much oxygen); and from the same material decomposing at the ordinary temperature of the room for from three days to three weeks. It has also been obtained from herring, twelve days in spring; from pike, six days in summer; from haddock, two months (BOCKLICH). Also from putrid mussel, sixteen days (BRIEGER); and from human as well as horseflesh. BRIEGER has obtained it from cultures of the bacteria of human feces on gelatin, and in small quantity in rather old cultures of the comma-bacillus on beef-broth; in larger quantity in cultures of the same germ on blood-serum. GARCIA found it in putrefying meat and pancreas, together with cadaverin and hexamethylenediamin. The diamin-production at 30° is considerable in twenty-four hours; reaches its maximum in three days. Putrescin appears on the first day. ROOS found putrescin in stools of one case of cholera and in two cases of diarrhœa or cholerin.

UDRÁNSZKY and BAUMANN in 1888 demonstrated the existence of putrescin and cadaverin in the urine of cystinuria. They found the total amount of the dibenzoyl compounds in the urine in 1888 to vary from 0.2–0.4 g. per day. Cadaverin made up about $\frac{2}{3}$ of this amount, and putrescin $\frac{1}{3}$ – $\frac{1}{4}$. GARCIA examined the same patient in 1892, and obtained, as an average of seven days only 0.064 g. of the dibenzoyl-compounds, which contained no cadaverin (!), only putrescin. With ordinary diet the average of 11 days was 0.027 g.; with cheese-diet an average of 8 days gave 0.136 g.; while a carbohydrate diet, an average of 7 days, gave 0.102 g. of the dibenzoyl-compound. In the feces of the same patient, on the contrary, UDRÁNSZKY and BAUMANN found in 1888 that putrescin constituted by far the greater quantity, while cadaverin formed but 10 to 15 per cent. GARCIA, in 1892, showed in the same patient the presence of only putrescin in the feces, no cadaverin. The feces contained on ordinary diet, an average of 11 days, 1.123 g.; on cheese-diet, average of 8 days, 1.3978 g.; on carbohydrate diet, average of 7

days, 0.741 g. of the dibenzoyl-compounds. BORISSOW, in 1894, again examined the feces of the same patient, and found as an average of four days 2.062 g. of the dibenzoyl-compounds, which contained only traces of cadaverin. It is, therefore, evident that diet and the intensity of intestinal decomposition influence the amount of excretion of diamins. The administration of salol and sulphur (MESTER), or intestinal lavage (U. and D.), has no effect on diamin-excretion. Normal feces, as well as the feces of various diseases, with the possible exception of cholera-stools, are free from diamins. It would seem, therefore, that these bases occur in cystinuria as the result of putrefactive changes going on in the intestines; becoming partly absorbed they appear in the urine. In two cases of cystinuria, reported by BRIEGER and STADTHAGEN, cadaverin was found almost solely present in the urine.

According to MESTER, the diamins are proportionate to the amount of cystin excreted, and therefore constitute a fixed symptom, the cause of which is the same as that of the cystinuria.

Although putrescin is recognizable on about the fourth day of the putrefaction, yet it does not occur in appreciable quantity until about the eleventh day. The amount that is formed increases as the putrefaction goes on, so that a considerable quantity may be obtained after two or three weeks. A very good source for the preparation of putrescin, cadaverin, and neuridin is gelatin which has been allowed to decompose in contact with water for some weeks. Neuridin is, apparently, formed first, but is soon replaced by the former two bases. In the process of extraction it is first obtained in the alcoholic mercuric-chlorid precipitate. For its separation from cadaverin and other accompanying bases, see Saprin, page 342.

From the urine of cystinuria it is best obtained by precipitation with benzoyl chlorid (Baumann's method). For this purpose about 1500 c.c. of urine are treated with 200 c.c. of sodium hydrate solution (10 per cent.), then 20 to 25 c.c.

of benzoyl chlorid is added, and the whole shaken till the odor of the latter disappears. The yellowish-white precipitate which forms may consist of insoluble phosphates, carbohydrates, polyatomic alcohols, and diamins. The cystin-compound is precipitated only in concentrated solutions. The precipitate contains from a half to two-thirds of the diamins present; it is filtered off, digested with warm alcohol, and the solution filtered. The alcoholic filtrate is concentrated and then poured into about thirty times its volume of cold water. The diamin-compounds then crystallize out. To separate the two diamins they are redissolved in just sufficient warm alcohol to effect solution, and this is then poured into about twenty times this volume of ether. The putrescin-benzoyl compound is thus thrown out of solution. The filtrate from this, on concentration, yields the cadaverin-compound. To isolate that portion of the diamins which remains in the original filtrate with benzoyl-cystin, it is acidulated with sulphuric acid and extracted with ether. The residue obtained on evaporating the ethereal solution is first neutralized with a 12 per cent. sodium hydrate solution, then mixed with three to four times its volume of the same solution. The precipitate which forms consists of the sodium compounds of benzoyl-cystin and the diamins. It is washed with sodium hydrate, and the two compounds separated by their different solubilities in water—the cystin-compound is readily soluble, that of the diamins insoluble. To purify the benzoyl-diamins they are dissolved in warm alcohol and precipitated with excess of water.

Putrescin (from *putresco*, to rot, to putrefy) is a water-clear, rather thin liquid which fumes in the air and has a peculiar semen-like odor, almost undistinguishable from that of cadaverin, and reminding one somewhat of the pyridin bases. It absorbs carbonic acid energetically from the air, without losing thereby the repulsive odor. The boiling-point of the free base, as ordinarily obtained, is about 135° . It is not decomposed by distillation with potassium hydrate, and is rather difficultly volatile with steam. With acids it forms beautiful crystalline salts. Putrescin unites with water, like ethylene-

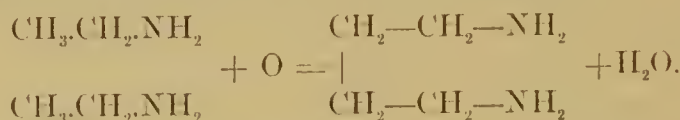
diamin, to form a hydrate, and this water can only be removed by distillation with metallic sodium. The perfectly anhydrous base boils at 156° – 157° , and then solidifies to plates (BRIEGER), which melt at 24° (UDRÁNSZKY and BAUMANN). The synthetic base boils at 158° – 160° , and melts at 23° – 24° (LADENBURG). Like cadaverin it is difficultly soluble in ether.

The constitution of putrescin has been determined by UDRÁNSZKY and BAUMANN (1888). They showed that the dibenzoyl-compound of putrescin was identical with that of the synthetic tetramethylenediamin and of the base which they found in the urine of cystinuria.

Putrescin, therefore, is tetramethylenediamin, a homologue of cadaverin, and its rational formula is :



The same authors (*Zeitschr. f. Physiol. Chem.*, **13**, 591) point out that diamins may possibly occur in putrefaction as the result of oxidation of monamins. Thus, putrescin might arise from methylamin according to the equation :



In a similar manner cadaverin might form from ethyl and propylamin. It is well known that in the decomposition of proteids in the presence of carbohydrates no aromatic compounds, as indol, phenol, tyrosin, etc., form. With reference to the formation of diamins GARCIA has shown that, in the presence of cane sugar, putrefying meat and pancreas yield from one-half to less than one-tenth as much diamins as when no sugar is present. A similar decrease of diamins in cystinuria is observed (page 325) when the patient is placed on a carbohydrate-diet.

The relation of diamins to cystinuria is as yet but little understood. BAUMANN and UDRÁNSZKY, accepting the intestinal origin of the diamins, supposed that these bases entered

into combination with cystin, protecting it against oxidation. When fed to dogs, however, the diamins are in part excreted as such, but no cystin appears. Again, cystin is not present in the feces of cystinuria. VERIGO considers cadaverin as a normal product of pancreatic digestion. GARCIA has shown in meat and pancreas putrefaction that diamin-formation begins on the first day, and reaches its maximum on the third day. Furthermore, meat and pancreas flasks inoculated with the feces of a cystin-patient produced an increased formation of diamins, thus confirming the view that diaminuria is the result of the activities of certain bacteria in the intestines.

It is possible that in diaminuria some other product is formed, which, when absorbed, combines with cystin and protects it against oxidation, so that it appears in the urine. Diaminuria and cystinuria certainly go hand in hand. GARCIA endeavors to account for the presence of diamins in cystinuria by the supposition that cystin, the normal product of the body, can undergo reduction and yield putrescin under the influence of special intestinal bacteria. Cystin, however, is not present in the feces in cystinuria, and when fed to dogs it merely serves to increase the amount of sulphuric acid eliminated. Moreover, the formula of cystin hardly permits of the derivation of diamins.

With reference to cystin it may be well to note that it has been met with, outside of cystinuria, only in a drunkard's liver (SCHERER); in beef kidneys (CLOETTA); in decomposing pancreas (KILZ); and in horse-liver (DRECHSEL).

Putrescin can be prepared synthetically, according to Ladenburg's method, by converting ethylene bromide into the cyanide and then reducing this by means of sodium in absolute alcohol. It is an isomer of ANGEL's dimethylethylenediamine.

On heating the concentrated aqueous solution of the hydrochlorid with potassium nitrite there is produced an oil, soluble in water, from which it can be extracted with ether. This oil, on treatment with phenol and sulphuric acid, gives Liebermann's nitroso-reaction, which would seem to show that

putrescin is not a primary diamine (butylenediamine), but is rather a secondary diamine (BRIEGER, II., 42). As a primary diamine it should take up, on repeated treatment with methyl iodide, six methyl-radicals; whereas, if it is a secondary diamine, only four methyl radicals can enter the molecule. Thus, to illustrate, methylamine, CH_3NH_2 (a primary amine), combines with three molecules of methyl iodide to form $(\text{CH}_3)_4\text{N.II}$. Similarly, dimethylamine, $(\text{CH}_3)_2\text{NH}$, requires only two molecules to form $(\text{CH}_3)_4\text{N.II}$. In the case of diamines, double this number of methyl-groups is required to effect complete saturation. As a matter of fact, BRIEGER, (III., 101), on treating putrescin with methyl iodide, has succeeded in introducing four, and only four, methyl radicals. From this, however, it does not follow that putrescin is not a primary amine, since cadaverine, an unquestioned primary diamine, yields a substitution-compound containing only two methyl-groups (see p. 336).

The tetra-methyl substitution-product of putrescin, $\text{C}_4\text{H}_8(\text{CH}_3)_4\text{N}_2$, can be distilled without decomposition. The free base crystallizes in long prisms. The hydrochloride forms small needles which are easily soluble; with phosphotungstic acid it gives a white crystalline precipitate, with phosphomolybdic acid a yellow crystalline precipitate, with picric acid needles. Potassium-bismuth iodide gives a brownish-red amorphous deposit, while the potassium-mercuric iodide forms prisms. Gold chloride yields difficultly, and platinum chloride easily soluble octahedra; aqueous mercuric chloride forms needles. The aurochloride has the formula $\text{C}_4\text{H}_8\text{N}_2\text{AuCl}_4$.

This tetra-methyl derivative of putrescin is enormously poisonous as compared with putrescin. The symptoms are the same as those produced by muscarine or nerin. They are: abundant salivation; dyspnoea—respiration at first increases, then decreases; contraction of the pupils; paralysis of the muscles of the limbs and trunk; increased peristaltic action of the intestines, ejaculation of semen, dribbling of urine, and, finally, violent clonic convulsions. In the case of

mice and guinea pigs the convulsions are prominent immediately after the injection of the poison.

PUTRESCIN HYDROCHLORID, $C_4H_{12}N_2 \cdot 2HCl$, forms long colorless needles, which are very easily soluble in water; difficultly so in dilute alcohol; entirely insoluble in absolute alcohol, and can thus be separated from cadaverin hydrochlorid. To accomplish this separation it is, perhaps, better to dissolve the mixture of the hydrochlorids in hot 96 per cent. alcohol. On cooling the solution thus obtained the putrescin salt crystallizes out, whereas that of cadaverin remains in solution. Putrescin hydrochlorid differs from cadaverin hydrochlorid in that it is not hygroscopic and can be exposed for days to the air without suffering any change on the surface of the crystals.

For the behavior of the free base and the hydrochlorid to alkaloidal reagents, see Table I. Putrescin is not toxic, though it possesses some marked physiological properties (see Cadaverin, page 335). According to SCHEURLEN, putrescin, like cadaverin, produces inflammation, suppuration, and necrosis. It is not poisonous to dogs (UDRÁNSZKY and BAUMANN). It is optically inactive.

The PLATINOCHLORID, $C_4H_{12}N_2 \cdot 2HCl \cdot PtCl_4$ (Pt = 39.16 per cent.), often appears under the microscope in the form of cholesterol-like plates. In the pure condition it appears as six-sided plates, which are superposed in layers. The crystals possess a splendid silvery lustre, and are rather difficultly soluble in cold water; less so in hot water.

The AUROCHLORID, $C_4H_{12}N_2 \cdot 2HCl \cdot 2AuCl_3 + 2H_2O$, crystallizes likewise in plates, which are difficultly soluble in cold water. It can, therefore, be readily separated from cadaverin aurochlorid, which is easily soluble in water. The water of crystallization can be driven off completely only at 110° (BRIEGER). According to BOCKLISCH, it loses this water on standing over sulphuric acid, or on heating at 100° .

The PICRATE, $C_4H_{12}N_2 \cdot 2C_6H_2(NO_2)_3OH$, is difficultly soluble, and crystallizes from a hot aqueous solution in needles; from hot aqueous alcohol, on cooling, in yellow plates. It begins

to brown at 230° , and on further heating becomes darker, till finally, at 250° , it decomposes with rapid evolution of gas (BOCKLISCH).

The CARBONATE is crystalline.

The MERCURY DOUBLE SALT is easily soluble in a large quantity of water, and can thus be separated from the cadaverin salt, which is difficultly soluble. From hot concentrated aqueous solution it crystallizes in needles.

The DIBENZOYL-PUTRESCIN, $C_4H_8(NHCOOC_6H_5)_2$, forms silky plates or long needles, which are more difficultly soluble in hot alcohol than those of the cadaverin-compound. From this solution it is reprecipitated by addition of water or ether. Its melting-point is 175° . It sublimes without decomposition.

CADAVERIN, $C_5H_{14}N_2$, is a diamine isomeric with saprin and neuridin, and, like the latter, its occurs very frequently in decomposing animal tissues. Twelve isomers of this composition are possible. Another isomer, gerontin (see next chapter), has been described by GRANDIS (1890). It is a very striking fact, that in ordinary putrefaction as cholin disappears the dianilins appear and increase in quantity according as the time of putrefaction is extended. It is also worthy of note that cadaverin appears in putrefaction before putrescin. It has been obtained by BRIEGER (1885) from human lungs, hearts, livers, etc. (hence the name), which were allowed to putrefy at the ordinary temperature for three days; from the same organs, and from horseflesh, after four months in a closed vessel at -9° to $+5^{\circ}$; from horseflesh after four months at 15° , together with cholin, and probably musearin (GULEWITSCH); from putrid mussel after sixteen days; from putrid egg and blood albumin. It seems to be a constant product of the growth of the comma-bacillus, irrespective of the soil on which it is cultivated.

BOCKLISCH has isolated it from perch and pike, six days in midsummer; from herring, twelve days in spring; from haddock, two months at a low temperature; from cultivations of Finkler and Prior's vibrio proteus on beef-broth, thirty

to thirty-five days at 37° to 38° (*Ber.* **20**, 1441). Cadaverin seems to be a constant product of the activity of the genus vibrio, inasmuch as it does not occur in cultures in which this genus is absent. Thus, it is not present in the excrements of healthy or typhoid patients; in cultures of Emmerich's bacillus, of Eberth's bacillus, and of the pyogenic bacteria. It is said to occur in cultures of the bacillus of hog cholera (v. SCHWEINITZ). OECHSNER DE CONINCK has found it in putrid jelly-fish (HUGOUNENQ). It is present with putrescin in the urine and feces of cystinuria ((UDRÁNSZKY and BAUMANN (1888), see page 325). The odor of cholera-stools and the breath of cholera-patients may be possibly due to cadaverin, although Roos has not been able to obtain diamins from the rice-water discharges of cholera. In one of four cases a small amount of a dibenzoyl-compound, crystallizing in small white plates and needles, and melting at 175° – 177° , was obtained. This corresponds with the putrescin-compound. No diamins were found in two cholera-urines. It would, therefore, seem that in Asiatic cholera diamins are not usually found in the feces of cholera, and since they are present in the feces and urine of cystinuria without bad results, it is evident that they cannot exercise any great action as intestinal poisons in cholera.

In a diarrhoea, where a coliform bacillus was present, Roos found both cadaverin and putrescin in the discharges, but not in the urine of one case. In another case cadaverin was, likewise, probably present. VERIGO has reported cadaverin from the intestinal contents of a woman with intestinal fistula. He would consider cadaverin as a normal product of pancreatic digestion.

BRIEGER was the first to show that diamins were absent from normal feces. BAUMANN and UDRÁNSZKY confirmed this observation with reference to man and the dog. The discharges of various diseases gave negative results except in typhoid stools, where a very small amount of dibenzoyl-compounds, melting at 140° , was found. Roos, in 1891, was able to find but two cases with diamins in the feces. In

one case of dysentery and malaria of tropical origin cadaverin was found, and in a case of cholera putrescin was found. It has also been obtained from caviar. LÖBSEN and ROKITSANSKY have reported it in bronchiectatic sputum. VERIGO has obtained it from pancreas extracts before putrefaction has set in; while GARCIA, from putrefying meat and pancreas isolates it together with putrescin and hexamethylenediamine.

Cadaverin occurs in the mercuric-chloride precipitate, from which it is isolated according to the methods given on pages 326 and 343. For its isolation and separation from putrescin by the use of benzoyl chloride, see page 327.

This base was at first ascribed to formula $C_5H_{16}N_2$, but subsequent researches led BRIEGER and BOCKLICH to the adoption of the formula $C_5H_{14}N_2$. In 1883, LADENBURG prepared, as the first step in the synthesis of piperidine, a base, pentamethylenediamine, possessing the same empirical formula as cadaverin, and later (*Ber.* 18, 2956) he showed the possibility of the identity of these two bases. This led to their direct comparison and the successful establishment of their identity. In fact, LADENBURG, as a crucial test of the identity, converted cadaverin into piperidine, and found the latter base to agree entirely in its chemical and physical properties with those of the natural alkaloid (*Ber.* 19, 2586). LADENBURG, however, observed one apparent difference between cadaverin and pentamethylenediamine, and that was in the composition of the mercury double salts. That of the former base, whether obtained from alcoholic or aqueous solution (BOCKLICH, *Ber.* 20, 1441), was found to combine with four molecules of mercuric chloride; whereas the double salt of pentamethylenediamine was found by LADENBURG to contain only three molecules of mercuric chloride. Subsequently he found that he had prepared this salt by mixing the aqueous solutions of the hydrochloride of the base and of the mercuric chloride in the molecular ratio of 1 to 4, and on using a larger excess of mercuric chloride he obtained a salt containing four molecules of mercuric chloride (*Ber.* 20, 2216). The complete identity of these two bases has, therefore, been established. The constitutional formula of cadaverin is, therefore:



Cadaverin can be prepared synthetically according to Ladenburg's method. For this purpose trimethylene bromid is converted into the cyanid, and this is then reduced by sodium in absolute alcohol.

Cadaverin forms a somewhat thick, water-clear, syrupy liquid, which possesses an exceedingly unpleasant odor, resembling somewhat that of coniin (piperidin) and of semen. When dehydrated with potassium hydrate it boils at $115^\circ\text{--}120^\circ$ (BRIEGER). It boils at 175° (BRIEGER, III., 98), and fumes in the air. The base eagerly absorbs carbonic acid from the air, and solidifies into a crystalline mass, the carbonate. It is volatile with steam, and can be distilled, without decomposition, even in presence of sodium or barium hydrate, or soda-lime. Neuridin, its isomer, decomposes under these circumstances. When heated with alcoholic potash and chloroform it does not give the isonitril reaction, nor does it give the characteristic odor of oil of mustard on treatment with carbon disulphid and mercuric chlorid. The absence of these reactions at first induced BRIEGER to conclude that cadaverin and putrescin were not primary amins, but LADENBURG (1885) showed that this conclusion was not justifiable. These two reactions are given by primary monamins, but in this case they are not given by cadaverin, a primary diamin. It is probable that this behavior holds true for all diamins.

Cadaverin is, undoubtedly, identical with the so-called "animal coniin," which has been isolated at various times from cadavers.

Cadaverin and putrescin were at first regarded as physiologically indifferent, but more recent investigations by SCHEURLEN, CRAWITZ, and others, show that both these bases are capable of producing strong inflammation and necrosis. According to BEHRING, in large doses it is poisonous to mice, rabbits, and guinea-pigs; it is not poisonous to dogs (UDRÁNSZKY and BAUMANN). Cadaverin is one of those substances which can set up suppuration in the absence of bacteria. In cholera

Asiatica the necrosis of the intestinal epithelium is quite common, and it would seem that this pathological change, as well as the muscular spasms and algidity, are due to the presence of these bases. It should be noted, however, that UDRÁNSZKY and BAUMANN failed to obtain any sign of intestinal irritation on feeding dogs enormous doses of cadaverin; and, moreover, ROOS (page 333) failed to find these bases in the feces of cholera. Besides these local effects, they prevent, even in small quantity, the coagulation of blood, and render it "laky." According to GRAWITZ, cadaverin seems to hinder the growth of bacteria. The cystitis observed in cystinuria may possibly be due to the presence of cadaverin and putrescin in the urine. Both bases are optically inactive.

When cadaverin is treated with methyl iodide, a base is obtained, the hydrochlorid of which gives with platinum chlorid a double salt, having the composition: $C_5H_{12}(CH_3)_2N_2 \cdot 2HCl.PtCl_4$. This new base, therefore, is cadaverin in which two atoms of hydrogen have been replaced by two methyl-radicals. The platinochlorid of this derivative forms long, clear red needles, which, unlike those of cadaverin, do not change their shape on repeated recrystallization. It is moderately difficultly soluble in water (BRIEGER, II., 41). Since cadaverin is a primary diamine it should combine with six molecules of methyl iodide to form a saturated compound. This, however, has not been obtained.

The HYDROCHLORID, $C_5H_{14}N_2 \cdot 2HCl$, crystallizes in beautiful, long deliquescent needles (BRIEGER). According to BOCKLISCH, it forms long, colorless needles or prisms; crystallizes from alcohol in plates, and is not deliquescent except on long standing. From 95 per cent. alcohol it crystallizes in short, pointed stellate prisms, which are not deliquescent (GULEWITSCH). On evaporation of an aqueous solution it forms very long prismatic crystals. It shows no circumpolarization. It possesses a slight bitter taste (GULEWITSCH). It is soluble in water, alcohol, alcohol-ether; but is insoluble in absolute alcohol, ether, etc. It can readily be separated from putrescin hydrochlorid by its solubility in 96 per cent.

alcohol (BOCKLISCH). The strictly pure base, as well as the hydrochlorid, does not give a blue color with ferric chlorid and potassium ferricyanid. For reactions of the hydrochlorid and of the free base, see Table I.

Cadaverin hydrochlorid on dry distillation decomposes into NH_3 , HCl , and piperidin, $\text{C}_5\text{H}_{11}\text{N}$. The latter is a well-known poisonous alkaloid which exists in the combined state in black pepper. It is not known whether this change, whereby the non-poisonous cadaverin is converted into a toxic base, can take place under the influence of bacteria during the process of putrefaction, or not. However, it does not seem improbable that this simple chemical change should be effected through the action of living organisms; for SCHMIDT has already shown that the almost physiologically indifferent cholin, when subjected to the action of the bacteria of hay-infusion, decomposes into a neurin-like base possessing a muscarin-like action, and under certain conditions it yields a base which in its action resembles pilocarpin.

The SULPHATE likewise forms beautiful, well-formed needles, and in its solubility corresponds to the hydrochlorid.

The PLATINOCHLORID, $\text{C}_5\text{H}_{11}\text{N}_2 \cdot 2\text{HCl} \cdot \text{PtCl}_4$ ($\text{Pt} = 38.08$ per cent.), crystallizes after some time, on the addition of platinum chlorid, to a not too concentrated solution of the hydrochlorid, in the form of long, beautiful orange-red needles (BOCKLISCH). Ordinarily it is obtained at first in long, dirty-red needles, which on repeated recrystallization become clearer and assume a form similar to that of ammonium platinochlorid. It forms chrome-yellow rhombic prisms which are short and octahedra-like. Variation in the crystalline form is observed here as in the case of the mercury-compounds. In polarized light they are strongly double refracting. It is very slightly soluble in cold water; can be recrystallized from hot water (BOCKLISCH). Its solubility in water at 12° is 1 to 113–114; at 21° it is 1 to 70.8 (GULEWITSCH). It is soluble in alcohol. It decomposes at 235° – 236° . It does not lose weight at 125° – 135° ; at 195° it begins to darken and melts with decomposition at 215° (GULEWITSCH).

The AUROCHLORID, $C_5H_{11}N_2 \cdot 2HCl \cdot 2AuCl_3$ ($Au = 50.41$ per cent.), crystallizes partly in cubes, and partly in long needles which at first possess a bright lustre, but under the desiccator soon effloresce and become opaque. It crystallizes from water, acidulated with hydrochloric acid, in plates or in large, long, very pretty orange-yellow flat prisms. On rapid crystallization bright platelets form (GULEWITSCH). The water of crystallization is completely removed on standing over sulphuric acid. It is very easily soluble, and melts at 188° (BOCKLISCH); 186° – 188° (GULEWITSCH).

The PICRATE, $C_5H_{14}N_2 \cdot 2C_6H_2(NO_2)_3OH$, forms yellow plates which are difficultly soluble in cold water. From hot water it crystallizes in long prisms, which melt at 221° with decomposition. When crystallized from 95 per cent. alcohol it forms long yellow needles, which are difficultly soluble in cold, more easily in hot 95 per cent. alcohol. It is insoluble, or very difficultly so, in absolute alcohol, and can be recrystallized from hot dilute alcohol.

Cadaverin hydrochlorid combines with mercuric chlorid, when the aqueous solutions of these two salts are mixed in the molecular ratio of 1 to 4, to form $C_5H_{14}N_2 \cdot 2HCl \cdot 3HgCl_2$. This salt can be recrystallized from hot water (LADENBURG). When an excess of mercuric chlorid is used the double salt has the composition $C_5H_{14}N_2 \cdot 2HCl \cdot 4HgCl_2$. This last salt melts at 216° (LADENBURG); at 214° (BOCKLISCH). It is difficultly soluble in cold water; easily in hot water at 21° (1–32.5, GULEWITSCH); from hot water it crystallizes in needles or plates (BOCKLISCH). On heating even on the water-bath it loses weight. At 125° – 135° it loses 18.33 per cent. of its weight, due to volatilization of mercuric chlorid (GULEWITSCH). As pointed out by BRIEGER, it is quite probable that other mercuric compounds exist than those mentioned. GULEWITSCH (1894) showed interesting polymorphism of the mercury salts of cadaverin. He inclines to the belief that it may form compounds with more than three or four molecules of mercuric chlorid. On heating mercuric chlorid is given off, and hence the varieties in form. This varia-

tion in form is, therefore, not necessarily due to impurities. When first obtained the cadaverin mercurchlorid forms warty aggregates of dark-brown prisms with pointed ends. By repeated recrystallization from water it eventually forms single or stellate rhombic plates, and on further crystallization very thin, elongated, six-sided, or triangular plates form.

The NEUTRAL OXALATE, $C_5H_{14}N_2 \cdot H_2C_2O_4 + 2H_2O$, was prepared by BOCKLISCH by adding a little less than the calculated quantity of alcoholic oxalic acid to the cadaverin. The precipitate may be recrystallized from hot dilute alcohol, when it is obtained in the form of needles, which melt at about 160° , and at the same time give off gas.

The ACID OXALATE, $C_5H_{14}N_2 \cdot 2H_2C_2O_4 + H_2O$, is made by bringing the neutral salt into alcoholic oxalic acid. It is soluble in hot dilute alcohol, and recrystallizes from it in quadratic plates, sometimes in glistening needles. It melts at 143° with decomposition. After it has been dried over sulphuric acid it loses, on being heated to 105° – 110° , one molecule of water (BOCKLISCH, *Ber.* **20**, 1441). The insolubility of these oxalates in absolute alcohol shows the fallacy of TAMBA'S distinction between ptomains and vegetable alkaloids. (See page 302.)

The DIBENZOYL-derivative, $C_5H_{10}(NHCO^6C_6H_5)_2$, crystallizes in long or small needles and plates, readily soluble in alcohol, difficultly so in ether, and insoluble in water; hence the alcoholic solution can be precipitated by addition of water or ether (separation from the putrescin-compound, see page 327). It melts at 129° – 130° ; at 130.5° – 131.5° (GULEWITSCH). It is not changed by boiling with dilute acids and alkalis; but boiling with concentrated hydrochloric or sulphuric acid for a long time finally breaks it up.

NEURIDIN, $C_5H_{14}N_2$, was the first diamine isolated from animal tissues (BRIEGER, 1883). It is one of the most common products of putrefaction, and as such has been obtained by BRIEGER from putrid horseflesh, beef, human muscle, five to six days; from haddock, five days in summer, from cheese, six weeks in summer; from gelatin, ten days at

53°; from decomposing human internal organs, three to eleven days; from cultures of the Eberth-bacillus, with mydin. BOCKLISCH has obtained it from perch, six days in summer; from barbel after three days in summer.

It has also been obtained from fresh eggs in the preparation of cholin by heating with baryta; and also from fresh brain by heating with 2 per cent. hydrochloric acid (BRIEGER, I., 57-61.). EHRENBERG (1887) found it in poisonous sausage and obtained it by growing a bacillus from this source on liver and meat bouillon.

Neuridine is almost invariably accompanied by cholin, and as the duration of putrefaction increases the latter gradually decreases in amount and yields a corresponding increase in trimethylamin, whereas the yield of neuridin increases from day to day. The amount of neuridin formed depends upon the nature of the organ employed in putrefaction. The greatest yield is obtained from gelatinous tissues, such as intestines; and especially from pure gelatin. On the other hand, such tissues as the spleen and liver yield but little.

Neuridin comes down in the mercuric chlorid precipitate (sometimes it occurs in the filtrate), and can then be isolated from the other bases present in a number of ways. One method is given under gadinin. Another convenient method of separation is to precipitate it from alcoholic solution by alcoholic picric acid. The pierate thus obtained is, for the purpose of further purification, recrystallized from absolute alcohol, then decomposed by extracting its acid solution with ether (to remove the picric acid) and evaporating the aqueous solution to dryness. The residue is now extracted with alcohol and the alcoholic solution precipitated by alcoholic platinum chlorid. The platinochlorid can now be recrystallized from hot water.

The free base, as obtained by the treatment of the hydrochlorid with moist freshly precipitated silver oxid, possesses an extremely repulsive odor, similar to that of human semen.

On evaporation of its aqueous solution it yields a gelatinous-like mass, and at the same time slowly decomposes. It

does not crystallize when evaporated in a vacuum, and decomposes even under these conditions. The same disagreeable odor is obtained when the hydrochlorid is warmed with potassium hydrate. BRIEGER (I., 24) regards this decomposition-product of neuridin as an oxidation-product of the original substance.

The free base is very readily soluble in water, but is insoluble in ether and absolute alcohol; difficultly soluble in amyl alcohol. It gives white precipitates with mercuric chlorid, neutral and basic lead acetates. When distilled with fixed alkali it yields di- and tri-methylamin, thus probably showing some relation to neurin, hence the name neuridin. It does not give Hofmann's isonitril-reaction, but it does not follow from this, as shown under cadaverin, that it may not be a primary diamin. It is isomeric with cadaverin, saprin, and gerontin.

The HYDROCHLORID, $C_5H_{11}N_2 \cdot 2HCl$, crystallizes in long needles which are extremely soluble in water and in dilute alcohol, but are insoluble in absolute alcohol, ether, benzol, chloroform, petroleum-ether, benzine, amyl alcohol, etc. Its insolubility in absolute alcohol may be used to effect a separation from cholin hydrochlorid. It can be recrystallized from slightly warm dilute alcohol. Although the pure salt is insoluble in the reagents just given, nevertheless, in the presence of other animal matter, it is dissolved in greater or less quantity, and hence can be obtained by the Stas-Otto as well as by the Dragendorff method. The crystals resemble urea in form. On heating very cautiously the salt sublimes, and at the same time appears to undergo a partial internal decomposition, inasmuch as many of the groups of needles in the sublimate are colored red or blue. For the behavior of the hydrochlorid with the alkaloidal reagents, see Table I.

Pure neuridin is not poisonous, but as long as it is contaminated with other putrefaction-products it possesses a toxic action similar to that of peptotoxin. This holds true for the other non-poisonous bases.

The PLATINOCHLORID, $C_5H_{11}N_2 \cdot 2HCl \cdot PtCl_6$, crystallizes in

beautiful flat needles. Recrystallized from hot water, it forms aggregations of small, clear, yellow needles. It is readily soluble in water, from which it is precipitated on the addition of alcohol.

The AUROCHLORID, $C_5H_{11}N_2 \cdot 2HCl \cdot 2AuCl_3$, is rather difficultly soluble in cold water (BOCKLISCH), and crystallizes on cooling of the hot, saturated solution in bunches of clear, yellow, short needles.

The PICRATE, $C_5H_{11}N_2 \cdot 2C_6H_2(NO_2)_3OH$, can be recrystallized from boiling water, in which it is very difficultly soluble, in the form of needles united in plumose groups. It is almost insoluble in cold water; less difficultly soluble in alcohol. It is not fusible, but begins to brown and give off yellow vapors at 230° , and carbonizes completely at 250° .

SAPRIN, $C_5H_{14}N_{22}$, was found in human livers and spleens after three weeks putrefaction (BRIEGER, II., 30, 46, 58). It occurs together with cadaverin, putrescin, and mydalein in the mercuric-chlorid precipitate. To separate these bases BRIEGER (1885) used the following process: The mercury salts were decomposed with hydrogen sulphide, the filtrate evaporated to dryness, and the residue then extracted with alcohol. The putrescin hydrochlorid is insoluble in alcohol, and is thus removed. The alcoholic solution was treated with platinum chlorid, which precipitated the greater part of the cadaverin. The mother-liquor, on concentration, yielded a mixture of the platinochlorids of cadaverin and saprin. Each successive crop contained more of the saprin double salt. The two kinds of crystals were now separated by means of a magnifying glass. The saprin platinochlorid thus obtained was finally purified by repeated recrystallization from water. The mother-liquor, after the removal of the saprin platinochlorid, contains the mydalein salt, which, on account of its solubility in water, crystallizes only on concentration, or on standing under a desiccator. The mercuric-chlorid filtrate contains some mydalein and the ptomain, which yields a platinochloride containing 28.40 per cent. platinum.

The free base is a diamin, and was first ascribed the formula $C_5H_{16}N_2$. It appears, however, to be isomeric with cadaverin and neuridin. The term saprin is derived from the Greek *σαπρός*, signifying putrid. It possesses a weak pyridin-like odor, and can be distilled with steam or with potassium hydrate without undergoing decomposition. In its reactions it behaves the same as cadaverin, except that it gives an amorphous precipitate with potassium-bismuth iodide, whereas cadaverin gives a crystalline precipitate. The free base gives an immediate intense blue color with ferric chlorid and potassium ferricyanid.

The HYDROCHLORID, $C_5H_{14}N_2 \cdot 2HCl$, forms flat needles which are not hygroscopic (distinction from cadaverin hydrochlorid. Its reactions are the same as those of cadaverin hydrochlorid. (See Table I.) It is, however, tinged slightly blue by a mixture of ferric chlorid and potassium ferricyanid, whereas the free base gives an intense blue. It differs from cadaverin in that it does not give the reddish-brown color with potassium bichromate and sulphuric acid. Again, it forms no aurochlorid; while, on the other hand, cadaverin hydrochlorid yields an easily soluble salt, crystallizing in splendid needles.

The PLATINOCHLORID, $C_5H_{14}N_2 \cdot 2HCl \cdot PtCl_4$, forms parallel, aggregated, pointed crystals, which are somewhat soluble in water, and are thus distinguished from cadaverin platinochlorid, which crystallizes in rhombs, and is difficultly soluble in water.

Physiologically, it is indifferent.

HEXAMETHYLENEDIAMIN, $C_6H_{16}N_2$. This compound was found by GARCIA in decomposing meat and pancreas mixture, seven days at 30° , by the benzoyl-chlorid method, together with cadaverin and putrescin. The dibenzoyl-putrescin is removed in the usual way by precipitating the alcoholic solution of the mixed benzoyl diamins with ether. The separation of cadaverin from the new compound is more difficult owing to the great similarity in the solubilities of the two compounds.

GARCIA succeeded in effecting a separation by dissolving the mixture in alcohol, and raising the temperature on a water-bath to 70° ; by gradual addition of water (50 volumes), taking care not to allow the temperature to rise above 70° , at which temperature the solution is left for twenty or thirty minutes; may be raised to 90° and kept there for one hour. The solution is then rapidly filtered through an asbestos-plug with the aid of a pump. On cooling, bright, long, crystalline plates and needles separate from the filtrate. The residue, dissolved in alcohol and again treated by this process, finally yields a residue of pure benzoyl-cadaverin. The crystals formed in the filtrate melt at 124.5° – 125° ; cadaverin-compound at 129° – 130° .

The dibenzoyl-compound in heating with equal parts of alcohol and concentrated hydrochloric acid on a water-bath for forty-eight hours is decomposed. The hydrochlorid is not deliquescent.

The PLATINOCHLORID, $C_6H_{16}N_2 \cdot 2HCl \cdot PtCl_4$ ($Pt = 37.01$), crystallizes from hot water on cooling in elongated, well-formed needles (rhombic system) of dark-orange color. Some were more than 1 cm. long. The crystallographic characters of the salt agree in all respects with those of the cadaverin-compound, with which it is therefore isomorphous. It is easily soluble in water, difficultly in strong alcohol.

Gold chlorid produces no precipitate in aqueous or alcoholic solution of the base. Picric acid gives a compound easily soluble in water and in absolute alcohol. It crystallizes in needles and plates; at 200° becomes brown, and at 210° it decomposes. In its behavior to gold chlorid it resembles saprin.

This base is not present in cystinuria.

A BASE, $C_7H_{10}N_2$.—Until very recently the nature of the basic substances which are formed as products of the alcoholic fermentation of sugar or molasses has been but little understood. KRÄMER and PINNER, in 1869, found in crude fusel oil a small quantity of a volatile base which they apparently identified with a collidin. This observation was confirmed by ORDONNEAU and others; and still more recently (January,

1888) MORIN has contributed an elaborate paper upon the bases formed during alcoholic fermentation. The portion of crude fusel oil which boils above 130.5° was extracted with slightly acidulated water, the acid aqueous solution thus obtained was made alkaline, and the oily bases which were thus set free were then distilled with vapor of water. The free bases were then dried over potassium hydrate and then subjected to fractional distillation. Three fractions were thus obtained, boiling respectively at 155° – 160° , 171° – 172° , and 185° – 190° . Only the second fraction, which boils at 171° – 172° , was studied, and was found to possess the formula $C_7H_{10}N_2$. Heated with concentrated hydrochloric acid, it is decomposed in part with the formation of ammonia. It combines with ethyl iodid to form a yellow crystalline compound which is soluble in water and alcohol, insoluble in ether. The hydrochlorid crystallizes in fine white needles, soluble in water and alcohol, and but very slightly soluble in absolute ether. The free base, as stated above, boils at 171° – 172° , is very soluble in water, alcohol, ether, etc. When pure it forms a colorless, strongly refracting, very mobile oil, which possesses a characteristic nauseating odor, but slightly resembling that of the pyridin-bases. Its density at 12° is 0.9826; toward litmus-paper the base shows no decided reaction. The platinochlorid is crystalline and is very soluble in water and alcohol, slightly soluble in ether. Potassio-mercuric iodid does not precipitate the aqueous solution of the free base, but in solutions of the hydrochlorid it gives a yellow flocculent precipitate, which soon crystallizes in long brilliant yellow needles. This reaction takes place readily in solutions of 1 to 1000, and only after some hours in solutions of 1 to 10,000; and is not given by the bases of the pyridic and quinolinic series. Mercuric chlorid produces an immediate flocculent precipitate in solutions of the base having a concentration of 1 to 1000, but requires some time to appear in 1 to 10,000. Phosphotungstic acid gives an immediate white precipitate even in a dilution of 1 to 10,000. Phosphomolybdic acid in solutions of the same strength yields a yellow precipitate.

The physiological action of this base has been examined by R. WURTZ, who found the lethal dose for rabbits, etc., to be about one gram per kilogram of body-weight. It produces stupor, paralysis, which at first appears in the rear extremities; the sensibility becomes diminished and the pupils are dilated and unresponsive to light; the rate of heart-beat is lowered, and the rectal temperature falls as low as 35° ; death follows a more or less prolonged coma.

TANRET obtained by the action of ammonia on glucose a number of bases, to which he applied the generic name of glucosins. One of these, having the formula $C_{14}H_{10}N_2$ ($C = 6$), corresponds in its formula and its general properties to MORIN'S base, $C_7H_{10}N_2$ ($C = 12$), and, in fact, the two bases are considered by TANRET to be identical.

It is interesting to note in this connection that alkaloidal bases have been found in petroleum by BANDROWSKI, and that similar basic substances have been detected by WELLER in paraffin-oil.

Most of the solvents in common use, such as alcohol, ether, chloroform, benzole, petroleum-ether, amyl alcohol, etc., have been shown at different times to contain basic pyridin-compounds, though ordinarily in very minute quantity. On the other hand, HARTINGER has found in some specimens of amyl alcohol as much as 0.5 per cent. of pyridin.

STISOTOXIN, $C_{10}H_{26}N_2(?)$, is a base isolated by NOVY in 1890 from cultures of the hog-cholera bacillus of Salmon (swine-plague of Billings). It is probably identical with the base obtained by v. SCHWEINITZ from the same germ, although the formula ascribed to it by him is $C_{14}H_{32}N_2$. The free base has not been obtained. The hydrochlorid forms a light-yellow syrup which shows no tendency to crystallize. It is soluble in water and in absolute alcohol, and is somewhat hygroscopic.

When heated with fixed alkali it gives off a strong aminodor, such as is perceived on evaporating the original culture-fluid, if it happens to be alkaline in reaction.

The platinochlorid is obtained by precipitation as a light,

flesh-colored, granular precipitate. It is readily soluble in water, from which it can be reprecipitated by addition of absolute alcohol. From aqueous solution, when allowed to evaporate slowly, it crystallizes in long, thick needles.

The mercurchlorid is thrown down from solutions of the hydrochlorid in absolute alcohol, by alcoholic-mercuric chlorid, as a heavy, white, granular precipitate. This readily dissolves on the addition of a small quantity of water, and can be perfectly reprecipitated by addition of absolute alcohol. On treatment with hydrogen sulphid it is readily decomposed, yielding the pure hydrochlorid.

The anurochlorid is very soluble in water and alcohol. From the alcoholic solution it may be partially precipitated by ether as a light-yellow, oily precipitate, which is adherent to the sides and bottom of the tube.

Physiological Action.—The base is toxic only in relatively large doses, as seen from the following experiment. About 100 milligrams, dissolved in a little water, were injected subcutaneously into a young rat. The animal was at first quiet, apparently unwilling to move. After some ineffectual attempts at jumping, it settled down to a recumbent position, and when placed on its side was unable to rise. Respiration was at first retarded, later increased, but toward the end was again very slow. Convulsive tremors shook the body at frequent intervals. The animal kicked vigorously. Reflexes were present almost to the end. As death approached, the red eyes whitened and took on a glazed, opaque appearance. Death resulted in one and a half hours. The animal was on its side, the feet extended. Post-mortem examination showed the heart arrested in diastole, lungs rather pale, stomach contracted, serum in thoracic cavity, subcuta pale and cedematous. Repeated doses of smaller quantities seem to confer a partial immunity to the action of the germ.



This base has long been known as a product of the oxidation of creatin and creatinin, but had never been met with

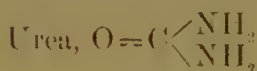
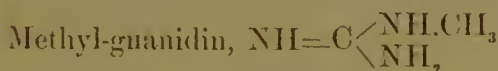
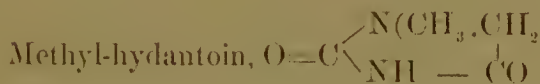
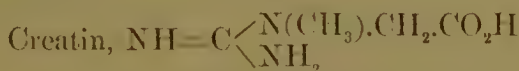
in animal tissues. BRIEGER in 1886 (III., 33) obtained it from horseflesh which was allowed to decompose in a closed vessel at a low temperature (-9° to $+5^{\circ}$) for four months. BOECKLISCH (*Ber.* **20**, 1441) isolated it from impure cultures on beef-broth of Finkler and Prior's vibrio proteus, containing ordinary putrefaction-bacteria, for twenty to thirty days at 37° - 38° . Vibrio proteus alone seems incapable of forming this base. The comma-bacillus, after some time (six weeks), partially decomposes creatinin with formation of a small quantity of methyl-guanidin (BRIEGER). The bacillus of anthrax likewise is capable of transforming creatin into methyl-guanidin.

It occurs in the mercuric-chlorid filtrate (BRIEGER), from which it is obtained, after the removal of the mercury by hydrogen sulphid, by precipitation with phosphomolybdic acid. The precipitate is decomposed with neutral lead acetate, and the filtrate from this, after removal of the lead by hydrogen sulphid, is concentrated, and then sodium picrate added. The resinous picrate precipitate is purified by boiling with much water, and, finally, it is recrystallized from boiling absolute alcohol. According to BOECKLISCH, it occurs in the mercuric-chlorid precipitate (not in the filtrate), from which it is isolated, after removal of the mercury and concentration of the clear filtrate, by precipitation with sodium picrate. The precipitate, containing cadaverin, methyl-guanidin, and creatinin, is boiled with absolute alcohol (cadaverin picrate is insoluble) and the alcoholic solution is then evaporated to drive off the alcohol and taken up with water. From this aqueous solution, after removal of picric acid, methyl-guanidin is precipitated by gold chlorid, whereas creatinin remains in solution.

This ptomain is identical with the synthetic methyl-guanidin (methyluramin), which can be readily obtained by boiling a creatin solution with mercuric oxide or with lead dioxide and dilute sulphuric acid (DESSAIGNES). The parent-substance of methyl-guanidin as it occurs in putrefaction is undoubtedly the creatin which exists preformed in the mus-

cular tissue. If such is the case, the bacteria engaged in its production must be considered as possessing an oxidizing action, since this base is prepared synthetically from creatin by oxidation. That creatin does not offer much resistance to the action of bacteria is shown in the fact that Friedländer's pneumonia-coccus, which possesses but small chemical powers, is capable of slowly but steadily decomposing creatin, yielding as one of the products acetic acid. STRECKER and ERLÉNMEYER, as well as BAUMANN, have shown that creatin, although a substituted guanidin, is not poisonous, but is readily converted into creatinin, which is a relatively toxic substance. On the other hand, guanidin and methyl-guanidin are quite violent poisons. This is, therefore, another instance in which a toxic substance is formed by the action of bacteria from a previously non-poisonous base (see page 369). According to LOSSEN, guanidin is formed, though in small quantity, in the oxidation of albumin.

The formulæ of these closely related substances are here given for comparison :



Methyl-guanidin forms a colorless, easily deliquescent mass, possessing a strong alkaline reaction. On heating with potassium hydrate it decomposes, and yields ammonia and methylamin. It is a highly poisonous base.

The HYDROCHLORID, $C_2H_7N_3.HCl$, can be obtained from the picrate by dissolving the latter in water acidulated with hydrochloric acid, and extracting the solution with ether to remove the picric acid. The colorless aqueous solution now on evaporation yields a thin syrup which crystallizes in vacuum to compact prisms. These are insoluble in alcohol, and give with platinum chlorid a double salt of monoclinic needles (HAUSHOFER) which are very easily soluble (1 part in about 7 parts of water, TATARINOW).

The AUROCHLORID, $C_2H_7N_3.HCl.AuCl_3$ (Au = 47.71 per cent.), forms rhombic crystals (HAUSHOFER) which are easily soluble in ether, more difficultly in water or alcohol; readily soluble (BRIEGER). It readily decomposes on heating in pure water, but may be recrystallized from water acidulated with hydrochloric acid. It melts at 198° .

The PICRATE, $C_2H_7N_3.C_6H_2(NO_2)_3OH$, comes down at first as a resinous precipitate, which when boiled with much water solidifies in the form of felted needles. It is very difficultly soluble in water, and can be purified by repeated recrystallization from boiling absolute alcohol—distinction from eadaverin. It melts at 192° .

The OXALATE, $(C_2H_7N_3)_2.H_2C_2O_4 + 2H_2O$, forms crystals which are easily soluble in water.

Physiological Action.—Methyl-guanidin as obtained from putrefying flesh is identical in its physiological action with the synthetic base. It has already been stated that the non-poisonous creatin is readily converted into the relatively energetic poison creatinin. The latter substance possesses a paralyzing action differing very much from its decomposition-product methyl-guanidin. This base is very poisonous, and the symptoms are marked by dyspnoea, muscle-tremor, and general clonic convulsions. BRIEGER has observed the following symptoms on injection of about 0.2 gram of methyl-guanidin into a guinea-pig: The respiration at once becomes more rapid, and in a few minutes abundant passage of urine and stool takes place; the pupils dilate rapidly to the maximum and cease to react. The animal is uneasy but motion-

less, though not exactly paralyzed. Respiration becomes deeper and more labored, the head moves from side to side, the extremities become gradually paralyzed; dyspnoea sets in, the animal falls on its side, and dies (twenty minutes) amid general clonic convulsions of short duration. Fibrillary twitchings of the trunk-muscles are observed only in the beginning. Post-mortem showed the heart to be stopped in diastole, the intestines filled with fluid, the bladder contracted, the cortex of the kidney hyperæmic, but the papillæ of the kidneys surprisingly pale.

MORRHUIN, $C_{19}H_{27}N_3$, was obtained by GAUTIER and MOURGUES (1888) from the mother-liquors of asellin on concentration of the platinum-containing liquid. This substance constitutes about one-third (0.07 per cent.) of all the bases found in cod-liver oil, and is named from *Gadus morrhua*, the ordinary codfish. The free base is an oily, very thick, amber-yellow liquid, the odor of which resembles somewhat that of syringa. It floats on water and partially dissolves; is more soluble in ether and in alcohol. The base is very alkaline, and is caustic to the tongue. It absorbs carbonic acid, and is non volatile. The salts of copper are precipitated by it, but the hydrate formed is not redissolved.

The hydrochlorid is very deliquescent. The gold salt forms a yellow precipitate, which readily dissolves on warming. The platinum salt, $C_{19}H_{27}N_3 \cdot 2HCl \cdot PtCl_4$ (Pt = 27.56 per cent.), crystallizes in barbed needles, which are quite soluble. (Separation from aselline, p. 352.)

Physiological Action.—The base possesses the property of exciting the appetite; it acts as a diaphoretic and above all as a diuretic. 0.029 gram given subcutaneously to a guinea-pig produced in two and a half hours a loss of 13.5 grams in the weight of the animal. The same effect is produced in birds. Strong doses (0.1 gram per kilogram) produce fatigue and hebetude.

A BASE, $C_{13}H_{20}N_4$, was obtained as early as 1868 by OSER, who observed its formation during the fermentation of pure cane-sugar by means of yeast. The hydrochlorid when dried in vacuo is said to form a white, very hygroscopic foliaceous mass, which soon becomes brown on exposure to air. At first it imparts a burning taste, which is soon replaced by a very bitter sensation.

A BASE corresponding to the formula $C_{17}H_{38}N_4$ was obtained by GAUTIER and ETARD from the mother-liquors of the platinochlorid of the base $C_5H_{13}N$. Very little is known, however, in regard to the general properties of this base, owing to the small quantity which could be isolated. This base and the one obtained by OSER from the yeast-fermentation of sugar, $C_{13}H_{20}N_4$, and asellin, $C_{25}H_{32}N_4$, are the only ptomaines thus far isolated which are known to contain four atoms of nitrogen.

The PLATINOCHLORID, $(C_{17}H_{38}N_4 \cdot 2HCl.PtCl_4)$ (Pt = 27.52 per cent.), is readily soluble, and crystallizes in needles which possess a light-yellow flesh color. When heated to 100° , it slowly decomposes, giving off a syringa-like odor.

ASELLIN, $C_{25}H_{32}N_4$, isolated by GAUTIER and MOURGUES (1888), together with five other bases from cod-liver oil. It is present only in small quantity in the oil. The name is derived from *Asellus major*, the great codfish. The free base is thrown down from the solutions of the hydrochlorid by the addition of alkali, in amorphous white flocules which are almost insoluble in water. It is almost colorless, but on exposure to the air becomes slightly green. It is not hygroscopic, and possesses a density of about 1.05. On heating it melts to a viscid yellowish fluid, possessing an aromatic odor; is non-volatile. Although almost insoluble in water, it imparts to it an alkaline reaction and a bitter taste. It is soluble in ether, more so in alcohol.

The salts are crystallizable, but are partially dissociated by the action of warm water. The hydrochlorid forms crossed or entangled needles which are quite bitter. The gold salt is

very reducible. The platinoclorid, $C_{25}H_{32}N_4 \cdot 2HCl \cdot PtCl_4$ ($Pt = 24.41$), is orange-yellow in color; soluble in warm water, insoluble in cold water (separation from morrhuin, p. 351), and is rapidly changed by boiling water. The mercury salt is precipitated in the cold; redissolves on heating, and then, on cooling, recrystallizes.

In large doses it produces fatigue, short and rapid respiration, and stupor. Three milligrams of the hydrochlorid kill a greenfinch in fourteen minutes.

MYDIN, $C_8H_{11}NO$, is a non-poisonous base which was obtained by BRIEGER in 1886 (III., 25) from the putrefaction of about two hundred pounds of human internal organs; and also in cultures of the Eberth bacillus on peptonized blood-serum. It occurs in the mercuric-chlorid filtrate, and is isolated from it after the removal of the mercury by hydrogen sulphide, by precipitation with phosphomolybdic acid. The gummy precipitate which is produced is decomposed on the water-bath with a solution of neutral lead acetate, and the filtrate on evaporation yields a colorless hydrochlorid, crystallizing in plates. It is purified by recrystallization of the picrate.

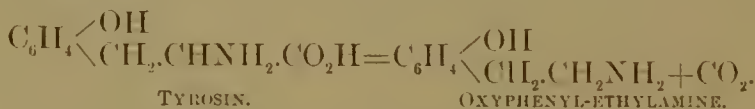
The free base is strongly alkaline, and possesses an ammoniacal odor. It is characterized by its strong reducing properties. The name mydin is derived from *μυδάω*, to putrefy. With platinum chlorid it gives, after a time, an extremely soluble salt; with gold chlorid, a precipitate of metallic gold. On distillation it is decomposed.

The HYDROCHLORID, $C_8H_{11}NO \cdot HCl$, crystallizes in colorless plates. It gives a blue color with ferric chlorid and potassium ferricyanid.

The PICRATE, $C_8H_{11}NO \cdot C_6H_2(NO_2)_3OH$, is obtained in broad prisms, which melt at 195° . It is the only salt suitable for manipulations.

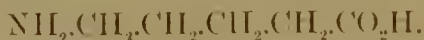
In describing NEXCT's collidin (page 312) it was stated that tyrosin might be looked upon as the source of that base. It would seem, however, to be more appropriately the parent

substance of mydin, inasmuch as it decomposes on being heated to 270° into carbonic acid and oxyphenyl-ethylamin, $C_8H_{11}NO$. The change that takes place can be represented by the equation :



A BASE, $C_5H_{11}NO_2$, was isolated by E. and H. SALKOWSKI (1883) from decomposing fibrin and meat. In its composition it is isomeric with betain anhydrid. It is extremely soluble in water, very diffiently so in alcohol, insoluble in ether, and possesses a semen-like odor and saline taste. The aqueous solution, which is not alkaline in reaction, yields on evaporation a stellate crystalline mass, which on standing over sulphuric acid becomes a white powder, which melts at 156° . It dissolves silver oxide, but not cupric hydrate, thus apparently indicating that it is not an amido acid. Moreover, it does not give a precipitate or blue coloration with copper acetate or ammoniacal silver nitrate. It thus differed from the then known amido-valerianic acids, its isomers. Recently, however (1891), GABRIEL and ASCHAN showed that δ -amido-valerianic acid agrees with this base in its reactions to copper nitrate. The gold salt of the synthetic base possessed the same composition as that of SALKOWSKI, and melted at 86° – 87° .

The identity of this base with δ -amido-valerianic acid (homopiperidinic acid) would seem to be established, and as such it is regarded. Its structure, then, is represented by



For its synthetic preparation see *Ber.* **24**, 1365 (1891). The base does not seem to possess toxic action.

The HYDROCHLORID, $C_5H_{11}NO_2 \cdot HCl$, forms colorless, stellate crystals, which are permanent in the air, and are extremely soluble in water, even in absolute alcohol.

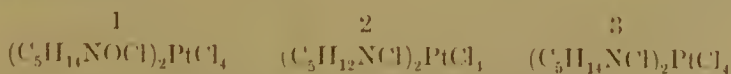
The AUROCHLORID, $C_5H_{11}NO_2 \cdot HCl \cdot AuCl_3 + H_2O$, is obtained on slow evaporation, as large, well-formed, beautiful

dark-yellow crystals. They are probably monoclinic, contain water of crystallization and melt at below 100° .

The PLATINOCHLORID gave on analysis results corresponding to the formula $(C_7H_{15}NO_2.HCl)_2PtCl_4$. This may possibly be due to the presence of some higher homologues of the base $C_5H_{11}NO_2$. It forms fine orange-yellow crystals, which are very difficultly soluble in alcohol, easily so in hot water, from which, on cooling, it crystallizes in beautiful plates.

CHOLIN GROUP.—The following four bases are closely related, and, indeed, starting from cholin, the oldest and best-known individual, the remaining bases can be readily prepared from it. Moreover, they can all be prepared synthetically according to methods that will be subsequently indicated. As cholin is the most prominent member, we have thought best to class these substances together as constituting the cholin group. It is very probable that mydatoxin and mytilotoxin, when their constitution becomes known, will be found to be homologues of certain members of this group.

NEURIN, $C_5H_{13}NO = C_2H_5N(CH_3)_3.OH$.—This substance was obtained and named thus by LIEBREICH (1865), who prepared it by boiling protagon for twenty-four hours with concentrated baryta. Previous to its discovery as a decomposition-product of protagon from the brain it was prepared synthetically by HÖFFMANN (1858) by treating trimethylamin and ethylen bromid with potassium hydrate or silver oxide. BAEYER (1866), by boiling an alcoholic extract of the brain with baryta water, obtained, on separation by three different methods, a base, or rather a mixture of bases, which, on analysis, gave results corresponding to the three formula:



Formula No. 3 was the one accepted by LIEBREICH for neurin, but, according to BAEYER, LIEBREICH's neurin salt is not

simple, but is a mixture of Nos. 1 and 2. He himself accepts formula No. 1 as the platinochlorid of neurin, and distinctly states (*Annal. d. Chem. u. Pharm.*, **142**, 323, 1867) that neurin is in composition trimethyl oxyethyl-ammonium hydroxid. And, according to him, cholin from bile, and sinkalin from white mustard, appear to be identical with neurin.

This nomenclature of BAEYER's was at first adopted by WURTZ and others, who showed that the oxyethyl base was identical with cholin and sinkalin. On that account STRECKER, in 1868 (*Annal.*, **148**, 79), suggested the restriction of the name cholin to the oxyethyl base, and to reserve the name neurin for the base whose platinochlorid is represented in No. 3, as originally was done by LIEBREICH. In 1869 LIEBREICH showed conclusively that pure protagon, when heated with baryta for twenty-four hours, yields a substance having the composition of the vinyl base:



The platinochlorid of this base crystallized in five-sided yellow plates, which, after a time, on exposure to the air, became cloudy; on treatment now with water a portion dissolved, and the solution was found to contain the oxyethyl base. Furthermore, he observed that when the alcoholic extract of the brain, from which all the protagon had been removed, is treated with baryta, only the latter, the oxyethyl base, is obtained. Finally, in 1870, WURTZ abandoned the use of the term neurin to designate the oxyethyl base, and returned to the name cholin, originally applied to the oxyethyl base by its discoverer, STRECKER. Nevertheless, the confusion in the use of these two terms continued to exist, and even at the present time it is the cause of no little misunderstanding. Thus, MARINO-ZUCO (1885), in his excellent researches on the genesis of ptomaines, applies the term neurin, following BAEYER's precedent, to the oxyethyl base, $\text{C}_5\text{H}_{15}\text{NO}_2$, which is really cholin, according to the proper nomenclature.

We have gone somewhat at this point in detail into the history and proper use of the terms neurin and cholin be-

cause of the confusion which is sure to arise if the distinction is not thoroughly borne in mind. The name neurin, then, should be used only to denote the vinyl base $C_5H_{13}NO$. It is trimethyl-vinyl-ammonium hydrate. On the other hand, cholin is applied to the oxyethyl base $C_5H_{15}NO_2$, which is trimethyl-oxyethyl-ammonium hydrate.

Neurin has been obtained by BRIEGER (1883) in the putrefaction of horse, beef, and human flesh for five or six days in summer. It also occurs in the commercial, so-called "neurin," together with cholin (BRIEGER, I., 34); in commercial 25 per cent. cholin (SCHMIDT). LIEBREICH obtained it in the decomposition of protagon by baryta. And BRIEGER (I., 60) also has isolated it along with cholin from fresh human brains, by boiling with baryta; but has not obtained it by digesting the brains on the water-bath with 2 per cent. hydrochloric acid. It has been found in putrid, and as result of this change poisonous, mushrooms (BERLINERBLAU, 1888).

The genesis of neurin is still rather obscure, and it is to be hoped that future investigations may shed more light upon the mysterious production of this highly poisonous base. Its occurrence in the brain together with cholin would seem to indicate that it is either derived from cholin by the removal of water, or that it exists together with cholin, partly replacing the latter in the molecule of protagon (lecithin), according to the hypothesis put forward by LIPPMANN (page 366). The question of its derivation from cholin by withdrawal of a molecule of water has already been subjected to an interesting experimental discussion. CH. GRAM attempted to explain the production of neurin and other muscarin-like ptomains as due to the dehydrating action of the acids employed in the methods of extraction, and, indeed, he claimed to have converted cholin platinochlorid, by heating with hydrochloric acid, into neurin. This statement has been disputed by BRIEGER, and by others, who showed that the platinochlorid of cholin, as well as the hydrochlorid, may be heated with fifteen or thirty per cent., or even concentrated hydrochloric acid, for six to eight hours on a water-bath, without any con-

version whatever (III., 15). That neurin may be obtained from cholin, at least by chemical processes, was shown by BAAYER, in 1866, who found that cholin chlorid, when heated with several times its volume of concentrated hydriodic acid and some red phosphorus, gave a compound $C_5H_{13}Nl_2$ which, on digestion with fresh, moist silver oxide, yielded a vinyl base identical with that previously obtained synthetically by HOFMANN, and now known as neurin. In HOFMANN'S method for the synthesis of neurin the trimethylamin ethylene bromid (see synthesis of cholin, p. 364) is treated with fresh moist silver oxide. SCHMIDT and BODE have shown that the iodine compound resulting from the action of hydriodic acid on cholin is the same as that formed by the action of the acid on neurin; and that on treatment with silver oxide it yields neurin. Cholin, therefore, may be readily changed into neurin. On the other hand, since neurin with hydriodic acid forms the same compound, by heating with silver nitrate cholin is formed. Hence neurin can be readily changed into cholin (SCHMIDT). On distilling cholin with water, or on dry distillation, a little neurin forms (NORTHAGEL). BRIEGER has tried, unsuccessfully, to bring about this dehydration by the putrefaction of pure cholin (I., 59). However, SCHMIDT and WEISS (1887) were more successful, and they found that cholin, as well as the hydrochlorid and lactate, is changed by the action of micro-organisms into the strongly poisonous neurin. Their results are given in full under cholin (see page 369). From what has been said it is evident that neurin can only arise from cholin, and this, as will be seen later, is derived from lecithin. Cholin may be kept dry or in aqueous solution for four months without change into neurin (SCHMIDT).

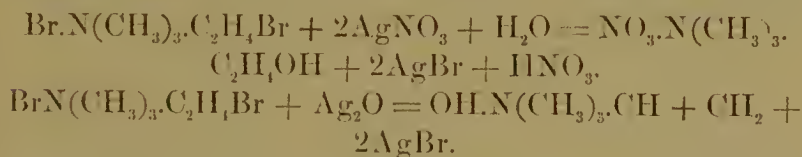
Neurin is almost invariably accompanied by cholin, from which, however, it can be readily separated by the difference in the solubilities of the platinochlorids. It occurs in the mercuric chlorid precipitate (and in the filtrate), and from this it can be obtained, after removal of the mercury, by precipitating the solution of the mixed hydrochlorids in absolute

alcohol by platinum chlorid. The platinumchlorids are then separated by recrystallization from water, since the neurin is difficultly soluble, while the cholin salt is readily soluble.

The free base possesses a strong alkaline reaction, and on contact with the fumes of hydrochloric acid it yields a cloud. According to LIEBREICH, the alkaline solution cannot be neutralized by passing through it carbonic acid.

With hydriodic acid neurin at 140° – 150° forms trimethylamin ethylene iodid, which on treatment with silver nitrate, as stated above, forms cholin, or on treatment with silver oxide regenerates neurin (SCHMIDT). This compound is the same as that prepared by BAEYER. It melts at 231° .

Fuming hydrobromic acid has no action on neurin at ordinary temperature, or at 100° ; but at 160° – 165° it yields trimethylamin ethylene bromid, which behaves with either nitrate or silver oxide as above (BODE and SCHMIDT). Thus:



Hypochlorous acid, likewise, breaks up the vinyl group in neurin to form a derivative of cholin, which with silver oxide yields iso-muscarin (BODE).

It decomposes readily on standing; more rapidly on heating into trimethylamin.

The CHLORID, $\text{C}_5\text{H}_{12}\text{N}.\text{Cl}$, is extremely poisonous, and crystallizes in fine hygroscopic needles. It is easily soluble in water and alcohol. By the action of hypochlorous acid it is changed to iso-muscarin.

The BROMID, $\text{Br.N}(\text{CH}_3)_3.\text{C}_2\text{H}_3$, is colorless, wart-like in form; hygroscopic and easily soluble in water and in alcohol, insoluble in ether. It melts at 193° (BODE).

The IODID, $\text{IN}(\text{CH}_3)_3.\text{C}_2\text{H}_3$, forms colorless, permanent needles; easily soluble in water; slightly in cold alcohol, easily in hot alcohol. When heated to 180° becomes yellow, and at 196° it melts (BODE).

The PLATINOCHLORID, $(C_5H_{12}N.Cl)_2PtCl_4$ (Pt = 33.60 per cent.), is difficultly soluble in hot water, and crystallizes in beautiful, well-formed, small octahedra belonging to the regular system. The crystals are always single. No twin crystals are observed. Sometimes the crystals contain water of crystallization, at other times they do not (BRIEGER, I., 33). It melts at 211° – 213° (SCHMIDT); 213° – 214° (BODE). According to LIEBREICH, it forms from an aqueous solution in five- or six-sided, heaped-up plates resembling urea nitrate, while from an alcoholic solution it forms needles, which on exposure to air become opaque, and are partially converted into the oxyethyl base—cholin. The difficult solubility, octahedral form, always single, and the melting-point distinguish it perfectly from the cholin salt.

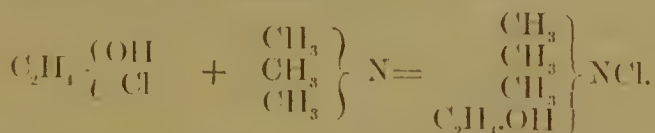
The AUROCHLORID, $C_5H_{12}N.Cl.AuCl_3$ (Au = 46.37 per cent.), forms flat prisms, which are difficultly soluble in hot water (BRIEGER). Dissolves easily, and can be purified by crystallization (LIEBREICH).

Physiological Action.—Neurin is exceedingly poisonous, even in small doses, and in its action it strongly partakes of the characteristic stamp of poisoning by muscarin. The injection of a few milligrams into frogs produces in a short time a complete paralysis of the extremities, with deadening of reflex excitability. Respiration stops first, while the rate of heart-beat gradually decreases till, finally, stoppage in diastole takes place. The injection of atropin at this point does away with the effect of neurin, so that the heart begins to beat again. Previously atropinized frogs, as a rule, withstand the action of the poison. Immediately after the introduction of this substance there can be observed a distinct period of exaltation, which, however, soon gives way to the characteristic stage of depression seen in the progressive slowing of the rate of heart-beat. Of the warm-blooded animals, cats seem to be much more sensitive to its action than mice, rabbits, or guinea-pigs. The symptoms seen in rabbits are profuse moistening of the nasal cavities and upper lip, which is succeeded by an intensely profuse salivation; later on there is noticeable an abundant secretion from the nasal mucous

membrane and from the eyes; the latter, however, ceases in a short time. The movements of the heart and of respiration are at first quickened and strengthened, but before long the paralytic effects produce a constant slowing and weakening, till finally complete cessation of both movements results. The decided dyspnoea observed gradually alters its character, and just before death the respiration is irregular and superficial. The heart, as in frogs, continues to beat after the respiratory movements have ceased, until finally it stops in diastole. Direct application of concentrated solutions of the poison to the eyes produces almost always a contraction of the pupil, while a similar but less constant contraction is seen when it is injected. The peristaltic action of the intestines is heightened to such an extent that continual evacuation takes place. Just before death, violent clonic convulsions occur. Atropin possesses a strong antagonistic action toward nemin, and the injection of even a small quantity is sufficient to dispel the symptoms just described.

CHOLIN, $C_5H_{15}NO_2 = C_2H_4OH.N(CH_3)_3.OH$.—This base is identical with the sinkalin of VON BABO, the bilineurin of LIEBREICH, and the neurin of BAEYER, MARINO ZUCO, and others. According to SCHMIEDEBERG and HARNACK, it is identical with LETELLIER's amanitin (agaricin), to which they assign, however, the formula $(CH_3)_3N.(CHOH.CH_3)OH$.

Cholin was first prepared, and so named by STRECKER, in 1862, by treating hog-bile with hydrochloric acid. It was prepared synthetically by WURTZ (1868) by direct union of ethylene chlorhydrin and trimethylamin. The reaction that takes place can be represented by the equation:



BAEYER (1866) obtained it by boiling an alcoholic extract of the brain with baryta water; and LIEBREICH, in 1869, showed that if the alcoholic extract, from which all the prota-

whereas pure protagon, on heating with baryta, yields neurin. It has been obtained from the yolk of eggs; from bile; from fresh brains (BRIEGER); from fresh eggs, blood, lungs, and hearts, and from lecithin (MARINO-ZUCCO); from human placenta (BÖHM); from the eye; from commercial neurin (BRIEGER); neurin was found in a specimen of commercial cholin (SCHMIDT); in commercial muscarin sulphate (NOTHNAGEL); from fresh as well as decomposing internal organs of the cadaver (BRIEGER, 1885); in fresh blood (neurin of MARINO ZUCCO and MARTINI); from herring-brine and decomposing pike, three days in midsummer (BOCKLISCH). It has also been isolated from cultures of vibrio proteus (BOCKLISCH, CARBONE) and of comma-bacillus (BRIEGER). EHRENBERG (1887) found it in poisonous sausage, and, by growing a bacillus obtained from this, on liver. GULEWITSCH has isolated cholin from horseflesh, putrefying at $15^{\circ}+$ for four months, together with cadaverin and probably muscarin.

Not only has cholin been met with in the animal tissues, but it has also been observed within the last few years to be very widely distributed in the vegetable kingdom, especially so in fatty seeds. Thus, it has been found (HARNACK, 1876) accompanying muscarin, in toadstool (*Agaricus muscarius*); in hops, and hence in beer (GRIESS and HARROW); in the seeds of *Trigonella*, in Indian hemp, areca- and earth-nuts, hemp seeds and lentils (JAHNS); in the seeds of white mustard, as a glycosid (VON BABO); in ergot (BRIEGER); in the germs of pumpkins and lupines (SCHULZE, *Zeitschr. f. Physiol. Chem.*, **11**, 365); in beech-nuts and morels (*Helvella esculenta*, *Boletus luridus*, *Amanita pantherina*, BÖHM); in flores sambuci (elder), and extracts of belladonna, hyoseyanus, ipecacuanha root and *Acorns calamis* (KUNZ), ipecacuanha root (ARNOLD), and *Scopolia Japonica* (SCHMIDT and HENSCHKE); in the sprouts and cotyledons of Soja beans (SCHULZE, 1888), in the fat from hog's bean, vetch, peas and lupines (JACOBSON, 1889); from the lecithin of lupine seeds (SCHULZE and STEIGER); and in Chicken leaves (*Myrtus chicken*, WEISS). According to LIPPMANN (*Ber.* **20**, 3206),

it is present together with betain, in the molasses from beet-root sugar. Cholin (RITTHAUSEN) and betain (BÖHM) exist together in cotton-seeds; hence, cholin occurs in the press-cakes from cotton-seeds (BÖHM). MAXWELL by extraction of cotton-seed cake with alcohol obtained about five times as much betain as cholin. With betain it occurs in worm-seed (*Artemisia Cina*, JAHNS); in sprouts of malt and wheat (SCHULZE and FRANKFURT). According to SCHULZE, and also RITTHAUSEN, cholin occurs with betain and another base in the seed of the vetch, and in peas with a base resembling betain. The two bases have also been found together in the roots and leaves of *Scapolia atropoides* by SIEBERT.

PARTHEIL found cholin, but not betain, in the seeds of *Cytisus laburnum*. KRESLING obtained it from the pollen of the fir, *Pinus sylvestris*.

SCHULZE and his pupils have shown that arginin, cholin, and xanthin bases occur in lupine sprouts. The same compounds with vernine occur in gourd sprouts, whereas in the sprouts of *Vicia sativa*, betain, cholin, and *guanidin*. Arginin on treatment with baryta yields urea. In the vetch, therefore, where arginin is absent, it is replaced by a derivative of urea, *guanidin*. Thus well-known animal products, as the xanthin bases, *guanidin*, tyrosin, leucin, are also met with in the vegetable kingdom.

Cholin may readily be prepared, after the method of DIAKONOW, from the yolk of eggs. These are extracted with ether, then with alcohol, and the extracts thus obtained evaporated, when the resulting residues are boiled with baryta for one hour. The filtrate, after the removal of the barium by carbonic acid, is evaporated and the residue is extracted with absolute alcohol. The alcoholic solution is now precipitated with platinum chlorid. BRIEGER (II., 55) has presented a method which is much simpler in its details and obviates the use of the expensive platinum chlorid. The tissues rich in lecithin, as yolk of eggs, brain, etc., are heated with concentrated hydrochloric acid for some hours on the water-bath. The insoluble residue is filtered off, and the filtrate, after neu-

tralization of the excess of free acid with carbonate of sodium, is evaporated. The residue is extracted with alcohol, and the alcoholic solution is precipitated with alcoholic mercuric chlorid. The precipitate thus obtained, on recrystallization several times from a large quantity of boiling water, yields the pure double salt of cholin.

If desirable, it can be made from pure lecithin, best prepared according to GILSON's method. Yolk of eggs is repeatedly shaken up with ether until the latter is colored only a faint yellow; the ether solution then distilled, the residue taken up in petroleum-ether and filtered. The filtrate, in a separatory funnel, is well shaken with 75 per cent. alcohol, and this is repeated several times with fresh alcohol. The alcoholic extracts are combined, allowed to stand for some time, then filtered and subjected to distillation to remove traces of petroleum ether. The solution is now set aside in a cool place for several days; the precipitate which forms consists of cholesterin, etc., and a little lecithin. The alcoholic solution is filtered by decantation, then decolorized by boiling with bone-black; rapidly evaporated at 50° – 60° to a syrupy consistency. This residue is extracted with ether, the solution filtered and evaporated. The lecithin thus obtained is almost perfectly pure, but contains traces of cholesterin. To purify it completely, it can be dissolved in as little absolute alcohol as possible, and set aside to precipitate in the cold— 5° to 15° .

Cholin may be prepared synthetically according to the method of Wurtz, or of Hofmann and Bode. In the latter ethylene bromid is heated with excess of alcoholic trimethylamin. The resulting bromine compound is treated with silver nitrate, filtered, and the filtrate heated on the water-bath for about eight days yields cholin nitrate. This is the easiest and cheapest method of preparation. It may be prepared from neurin (page 358).

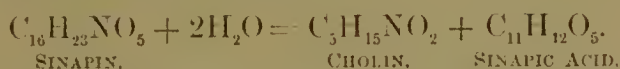
In regard to the genesis of cholin the preponderance of testimony goes to show that it is derived from the decomposition of lecithin, which, according to the researches of DIAKONOW and others, is one of the most widely distributed com-

pounds, occurring in greater or less quantity in all of the animal tissues. Lecithin, which is a complex ester (STRECKER, HUNDESHAGEN, GILSON), decomposes under the action of acids and alkalis into a base (cholin), glycerin, phosphoric acid, and fatty acids (stearic, oleic, palmitic, etc.). GILSON has shown that dilute sulphuric acid slowly decomposes lecithin, forming cholin, which, after a few days, disappears; on the other hand, sodium hydrate, in even 1 per cent. solution, rapidly decomposes it. This change is undoubtedly accomplished in a similar manner through the agency of bacteria. BRIEGER (II., 17) is inclined to believe that cholin exists preformed in the various tissues, inasmuch as he has been unable to obtain it from the brain, which is rich in lecithin, by boiling with 2 per cent. hydrochloric acid. (See SCHULZE, page 367.) Prolonged heating with concentrated hydrochloric acid was necessary in order to obtain any cholin from the brain. This result of BRIEGER'S is somewhat at variance with that of MARINO-ZUCO (see *Relazione*, etc., pages 29, 30, and 38), who obtained from 25 grams of lecithin, by the method of Stas, a small quantity of the aurochlorid of a base, while from a similar amount he obtained more relevant quantities by the method of Dragendorff.

The occurrence of cholin in the vegetable kingdom would be inexplicable to us at present were it not that we now know of the existence of lecithin-like bodies in plants, from the decomposition of which substantially the same products are obtained as from the lecithin obtained from the animal tissues. The existence of such a body in plants was first predicted by SCHEIBLER in 1870, who was led to this conclusion in his celebrated study of beet-root sugar, because of the presence of oleic acid, glycerin, phosphoric acid, and betain, as well as cholesterin, in the beet-root extracts. This hypothesis was confirmed by HOPPE-SEYLER, who, in 1879, found a lecithin-substance in yeast. SCHULZE found a similar compound in the cotyledons of lupine, while JACOBSON observed its presence in mustard-seeds, in fenugreek-seeds, in maize and wheat, in the fat from beans, peas, vetch, and lupines. HECKEL

showed its presence in globularia, and LIPPMANN has found it in beet-root. According to HOPPE-SEYLER, this lecithin-like substance exists in all vegetable cells undergoing development. SCHULZE and LIKIERNIK (1891) were the first to prepare lecithin in a pure condition from plants. It was found to possess the same properties and yield the same decomposition-products as lecithin from animal tissues. Up to the present time lecithin has always been supposed to contain a radical, which gives rise to cholin on saponification, as an essential component, while on the other hand the fatty acids entering its molecule are well known to be replaceable by one another. Thus we may have a di-stearin lecithin as well as a di-olein lecithin. The existence of several lecithins in the yolk of eggs has been recognized for some time, and according to SCHULZE and LIKIERNIK this is also true of the lecithins in plants. Recent observations of LIPPMANN (*Br.* 20, 3206) show that the above basic radical, hitherto regarded as constant in lecithin, may possibly be capable of replacement by other similar radicals. He found on saponifying with baryta two different specimens of lecithin, both obtained from beet-root, that while one of them yielded oleic acid, glycerin, phosphoric acid, and betain; the other lecithin gave oleic acid (and some other fatty acids), glycerin, phosphoric acid, and cholin, with no betain—at least not in isolable quantity. This remarkable difference has led LIPPMANN to suggest an explanation which, while it may not be the correct one, nevertheless possesses a high degree of probability. According to him, the lecithin molecule may contain interchangeable basic radicals in the same manner that it contains interchangeable acid radicals. This view is supported not only in the case of beet-root, where cholin and betain exist together, but the same two bases have been observed in cotton-seed. A similar co-existence was observed in the toad-stool (*Agaricus muscarinus*), in which cholin and muscarin were found. And, lastly, the same condition holds true probably for mytilotoxin and betain, which were shown to be present together in poisonous mussels.

Lecithin cannot always be regarded as the source of cholin in plants, since this base is known to occur as a glucosid in the seeds of white mustard. The sinapin decomposes according to the equation :



According to SCHULZE (1891), the cholin which is isolated from pea- and vetch-seeds, exists preformed in the seeds, and does not result from lecithin by the process of extraction. This is also probably true with reference to cotton-seed cake. The condition in which betain exists is not determined.

The protoplasm itself is another possible source of cholin as well as of other nitrogenous bases, as xanthin, etc. We know from DRECHSEL's brilliant investigation (1890) that casein on treatment with hydrochloric acid and stannous chlorid yields ammonia, amido acids, and organic bases—*lysatin*, $\text{C}_6\text{H}_{13}\text{N}_3\text{O}_2$, and *lysatinin*, $\text{C}_6\text{H}_{11}\text{N}_3\text{O}$ —homologues of creatin, $\text{C}_4\text{H}_9\text{N}_3\text{O}_2$, and creatinin, $\text{C}_4\text{H}_7\text{N}_3\text{O}$. From lysatinin urea can be readily obtained by treatment with baryta. Subsequently, SIEGFRIED (1891) showed that vegetable protoplasm (conglutin from lupine) when treated in the same way yields similar products. The two bases have since been prepared from gelatin by FISCHER; from horn by HEDIN. Later, SCHULZE demonstrated that the base, *arginin*, $\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$, is formed in lupine sprouts at the expense of the proteids present, and he pointed out that this base is probably related to lysatin, from which it differs only by NH (see next chapter). By the decomposition of horn substance with stannous chloride and hydrochloric acid HEDIN obtained arginin. SCHULZE and LIKIERNIK, by treatment with baryta, have converted arginin, the same as lysatinin, into urea.

DECOMPOSITIONS OF CHOLIN.—BAEYER (1866) succeeded in converting cholin into neurin by a purely chemical process. This was accomplished by heating cholin chlorid with concentrated hydriodic acid and red phosphorus in a sealed tube at 120° – 150° , whereby the compound $\text{C}_5\text{H}_{13}\text{N}_1\text{I}_2$

was formed. Fuming hydrobromic acid heated to 160° – 170° may also be employed. The iod-iodid of cholin thus obtained, on treatment with moist silver oxid gave a base, the platinochlorid of which corresponded to the formula $(C_5H_{12}NCl)_2PtCl_4 + H_2O$. This double salt, according to BAEYER, is readily soluble in water, and gives reactions similar to cholin. Although BAEYER is emphatic in his assertion that this is the vinyl compound (neurin) formed from the oxy-ethyl base (cholin), yet it seems that there is room for doubt in regard to the interpretation of his results. Thus neurin platinochlorid is difficultly soluble in water, contrary to the behavior of the platinochlorid obtained by him. On the other hand, cholin platinochlorid is easily soluble in water, and it would seem, therefore, that BAEYER has not converted cholin into neurin, but rather has regenerated cholin from its iod-iodide. If such were the case, we would expect that the iod-iodid of neurin, $C_5H_{13}NI_2$, which has the same composition as the corresponding derivative of cholin, would yield, on treatment with silver oxide, the oxy-ethyl base. BAEYER has apparently not been able to effect this change, since he holds that the vinyl base may be prepared from the oxy-ethyl, but that the reverse, the preparation of the oxy-ethyl base from the vinyl compound, cannot be accomplished. This has been successfully accomplished by SCHMIDT. Neurin can be changed into cholin, and *vice versa* cholin can be changed into neurin (page 358).

Whether the change described by BAEYER takes place or not, it is, nevertheless, certain that cholin does not readily give up a molecule of water, and thus become converted into neurin. CH. GRAM announced, in 1886, that cholin chlorid and lactate, on heating on the water-bath with dilute hydrochloric acid, decompose, and that this conversion into the vinyl base was easy and complete when the aqueous hydrochloric acid solution of cholin platinochlorid was heated for five or six hours on the water-bath. In this way GRAM endeavored to explain the formation of neurin as due to the action of acids upon cholin, but BRIEGER has shown

that the platinum salt of cholin, as well as its hydrochlorid, can be heated with fifteen or thirty per cent., or even concentrated, hydrochloric acid for six or eight hours without undergoing any change into neurin, thus disproving the results obtained by GRAM. E. SCHMIDT and WEISS have independently confirmed BRIEGER's observations in regard to the resistance of cholin to decomposition by acids. SCHMIDT has gone further, and has shown by an examination of GRAM's original preparations that it was cholin, and not neurin. GULEWITSCH (1894) was likewise unable to split up cholin by acids into neurin. Cholin, therefore, is not decomposed by acids into neurin. What the action of acids has failed to do is probably accomplished through the agency of bacteria. SCHMIDT found that cholin chlorid, when allowed to stand with hay infusion, or with dilute blood, for fourteen days at 20° – 30° , decomposed almost entirely, yielding large quantities of trimethylamin and a base, the platinochlorid of which resembles in form and solubility the double salt of neurin and possesses a similar physiological action. When allowed to decompose for ten days at 30° – 33° neither cholin nor neurin was present. Cholin lactate in hay infusion developed an odor of trimethylamin in twelve hours, but at the end of fourteen days a good deal of cholin was still present. In this case no neurin was present, but instead a homologous base was found, which can be obtained synthetically by the action of trimethylamin on allyl bromid. According to MEYER, of Marburg, this base does not possess the muscarin-like action of neurin, but resembles more closely pilocarpin.

The decomposition of cholin by putrefaction into neurin, a highly poisonous base, may explain the production of poisons in foods.

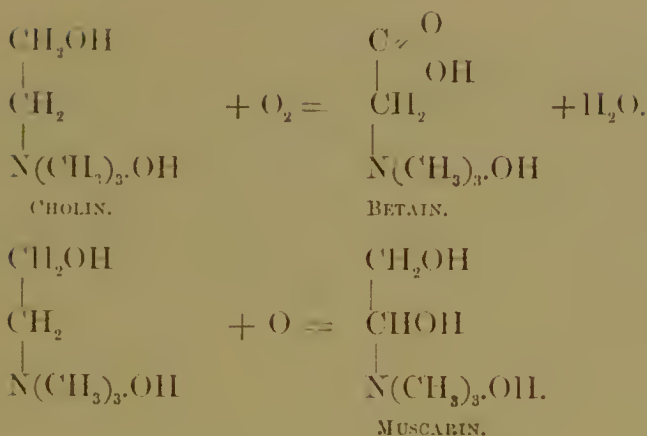
BRIEGER (I., 59) had unsuccessfully tried to transform cholin into neurin by putrefaction. He observed that the cholin decomposed with extreme slowness, even when the putrefaction was carried on at a higher temperature, yielding only trimethylamin. WURTZ (1868) showed that dilute solutions of free cholin can be heated to boiling without any

perceptible decomposition. Concentrated solutions, however, decompose with the formation of trimethylamin and glycol, $C_2H_4(OH)_2$ (see page 306). The decomposition of cholin was studied somewhat by MAUTHNER (1873), who confirmed WURTZ's observation that cholin was scarcely decomposed by boiling water, and he showed that when exposed to the action of decomposing blood it yielded trimethylamin. The results obtained by K. HASEBROEK (*Zeitschrift f. Physiol. Chem.*, **12**, 151, 1888) deserve special mention at this place. He carried on the putrefaction of very dilute solutions of the chlorid of cholin in the presence of little or no oxygen in Hoppe Seyler fermentation-flasks. Sewer slime, because of its strong fermentative properties, was used to induce the putrefaction, and calcium carbonate was added to neutralize any acidity that might develop during the fermentation.

The fermentation, as shown by the evolution of gases, lasted for about three months. The total quantity of gas given off was about one litre from 1.17 grams cholin chlorid. The gases consisted almost entirely of carbonic acid and marsh gas. No hydrogen was evolved. When the fermentation ceased the flask was opened and several cubic centimetres of the almost neutral clear liquid were injected under the skin of a rabbit without producing the least effect.

This liquid distilled with alkali gave methylamin and ammonia. What is remarkable about this experiment was the total absence of the higher amines—as, for instance, trimethylamin, which has been observed so many times as a decomposition product of cholin. The absence of any poisonous base, as neurin, was probably largely connected with the absence of oxygen.

Free cholin ordinarily forms a strongly alkaline syrup which combines readily with acids to form salts, most of which are deliquescent. By oxidation it is converted into betain (see page 376), and on treatment with concentrated nitric acid it gives rise to muscarin (see page 380). These reactions can be represented by the equations:



By the action of dilute nitric acid cholin is converted almost wholly into a base the platinochlorid of which is efflorescent, and forms large, bright crystals grouped in bunches. It corresponds to the formula $(\text{C}_4\text{H}_{10}\text{N}_2\text{O}_3\text{Cl})\text{PtCl}_4 + 2\text{H}_2\text{O}$ (SCHMIEDEBERG and HARNACK).

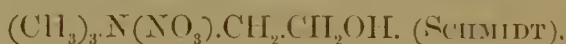
NOTHNAGEL has isolated the same compound, and, on attempting to convert the platinum salt into the gold compound, he obtained the aurochlorid of trimethylamin. The nature of this base, which is formed also in small quantity on oxidation with concentrated nitric acid, is uncertain.

According to MAUTHNER, cholin resembles the caustic alkalis in its action. Although putrefying blood decomposes it into trimethylamin, yet, when present in the proportion of 1.4 per cent., it is said to arrest putrefaction. A 1 to 2 per cent. solution is said to dissolve fibrin or coagulated albumin on boiling.

NOTHNAGEL has shown, contrary to ARNDT, that cholin cannot be distilled unchanged with baryta water. It is decomposed into trimethylamin. On distillation with water it yields a few drops of an aldehyde body, a little neurin (?), and trimethylamin. On dry distillation of cholin it yields also a little of the aldehyde body, a little neurin (?), and chiefly cholin. The latter probably results from recombination in the distillate of the trimethylamin, ethylene oxid, and water.

The free base, as well as the carbonate, is dimorphous and forms thin plates or long needles.

The CHLORID, $C_5H_{11}NO.Cl$, is easily soluble in water and in absolute alcohol (separation from neuridin hydrochlorid and from betain). It crystallizes over sulphuric acid to needles which readily deliquesce in the air. Potassium mercuric iodid produces in solution of the chlorid a crystalline precipitate. The nitrate possesses the formula :



The BROMID, $Br.N(CH_3)_3.C_2H_4OH$, forms rather long, colorless rhombic plates when ether is added to an absolute alcohol solution of the salt. It deliquesces very rapidly in the air and is decomposed by sunlight, changing color to violet and brown (NOTHNAGEL).

The IODID, $IN(CH_3)_3.C_2H_4OH$, can be crystallized in the same way as the bromid. It is less deliquescent and is turned yellow by sunlight (NOTHNAGEL).

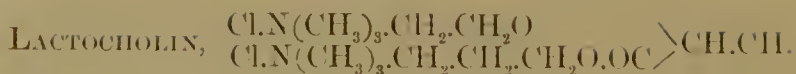
The PLATINOCHLORID, $(C_5H_{11}NO.Cl)_2PtCl_4$ (Pt = 31.64 per cent.), presents an interesting case of trimorphism. It crystallizes in monoclinic plates (RINNE) which are easily soluble in water, insoluble in alcohol; also in characteristic superposed plates, sometimes in the form of orange-red flat prisms (BRIEGER). From a warm saturated solution containing 15 per cent. alcohol it crystallizes in yellow regular octahedra containing one molecule of water of crystallization, from aqueous solutions in six-sided plates (JAHNS); from aqueous solution on slow evaporation it forms plates, clinorhombic plates, or needles (HOPPE-SEYLER) which are anhydrous. When rapidly crystallized it forms prisms (HUNDESHAGEN, JAHNS, SCHULZE); and if the solution is concentrated the prisms are very thin, almost needles. According to SCHULZE, it sometimes forms beautiful orange-red, chiefly six-sided plates. It is easily soluble, forming splendid, very large, red, superposed monoclinic plates; may crystallize from cold saturated aqueous solution in large, prismatic, or needle-shaped crystals (SCHMIDT). JAHNS maintains that the plates and prisms be-

long to the same system; while HUNDESHAGEN holds that they are distinct. Instead of the salt presenting an instance of trimorphism as first stated by HUNDESHAGEN, it would seem that but two forms occur—anhydrous monoclinic and octahedra with one molecule of water of crystallization. It contains always more or less water of crystallization, which it does not give up completely over sulphuric acid, but only at 110° (BRIEGER). The natural platinochlorid becomes strongly electric on rubbing, whereas the synthetic cholin double salt does not become electric. It melts at 225° with effervescence (JAHNS); at about 238° (PARTHELL); at 233° – 234° (BODE); at 232° – 233° , and more often at 240° – 241° (SCHMIDT), with marked effervescence. The solubility, crystalline form, and melting-point render it wholly different from neurin.

The AUROCHLORID, $C_5H_{14}NO.Cl.AuCl_3$ ($Au = 44.48$ per cent.), is crystalline, and is difficultly soluble in cold water, but can be recrystallized from hot water or from boiling alcohol. It forms prisms, or gold-yellow long needles, which are very easily soluble in hot water and alcohol (LIPPMANN). It can be separated from neuridin aurochlorid by its solubility in water (BRIEGER). On heating, the gold salt melts to a brown liquid (SCHULZE), and decomposes at 264° . It melts at 244° – 245° (SIEBERT, JAHNS); at 245° – 246° (SCHMIDT).

The PICRATE, $C_5H_{14}NO.OC_6H_2(NO_2)_3$, forms long, broad needles which are more easily soluble than neuridin picrate, and hence can be separated by recrystallization. It is more easily soluble in alcohol than in water.

The MERCUROCHLORID, $C_5H_{14}NO.Cl.6HgCl_2$, is extremely difficultly soluble even in hot water. On this account the mercury salt is very convenient for the separation of cholin from accompanying bases. It is very difficult to separate it from the cadaverin mercuriochlorid on account of the similarity in solubilities. GULEWITSCH secured a separation by fractional precipitation and fractional solution. It forms small, short, cross shaped prisms. Its solubility in water is 1:37.4 at 21° (GULEWITSCH).



This compound was obtained by SCHMIDT by heating an aqueous solution of cholin lactate on a water-bath for six days. The platinochlorid forms long prismatic crystals with roof-shaped ends. It is easily soluble in water; difficultly in alcohol. It has two molecules of water of crystallization, and melts at 220° – 221° . The platinum compound on decomposition with hydrogen sulphid or potassium chlorid, and precipitation with gold chlorid, yields, instead of a gold salt, the aurochlorid of cholin. All attempts to obtain the aurochlorid of lactocholin failed. Lactocholin is formed from ethylidene, and not from ethylene lactic acid (NOTHNAGEL).

Oxy-iso-butyro-cholin is produced in the same way as lactocholin. The platinochlorid has the same form and melting-point, 221° . The two molecules of water of crystallization are difficultly driven off. The gold-salt is not obtainable on account of reversion to cholin. The formula corresponds to that of lactocholin (NOTHNAGEL).

Oxy-valero-cholin is also prepared the same as lactocholin. The platinum salt crystallizes in long compact needles, containing two molecules of water of crystallization, which are rather easily soluble in water, and melt at 223 – 224° (NOTHNAGEL). Its composition also corresponds to that of lactocholin.

Oxy-acetic, ethylene lactic, and salicylic acids do not form anhydrid compounds. The above compounds result from the union of two molecules of cholin and one molecule of acid with elimination of two molecules of water. The platinum salts of all three anhydrides belong to the same system; all have two molecules of water of crystallization, difficultly expelled at 100° . The melting-point is about the same in all. They do not yield gold salts. The free anhydrides are not permanent.

Acetyl-cholin. The gold salt of this compound was studied first by BAEYER, later by NOTHNAGEL. BAEYER obtained the

acetyl compound by the action of acetyl chlorid on cholin chlorid in the cold, but NOTHNAGEL did not succeed in introducing the acetyl group short of 100° . The gold salt is anhydrous, tree-like in form, and melts at 154° – 155° . On decomposition with hydrogen sulphid it yields cholin. The platinochlorid crystallizes in small anhydrous needles which melt at 223° – 224° .

Benzoyl-cholin is formed by heating dry cholin chlorid on a water-bath with benzoyl chlorid. It forms a platinum salt crystallizing in fine thread-like needles, which melt at 206° . The gold salt forms light-yellow flat needles which are permanent in the air and melt at 183° . The hydrogen in the hydroxyl group of the oxyethyl is, therefore, easily replaced by acid radicals (NOTHNAGEL).

Physiological Action of Cholin.—Cholin was regarded for a long time as physiologically inert, but this belief was set aside by GAEHTGENS (1870), who showed that, when given in large quantity, it possessed a marked toxic action; 0.59 gram producing almost instantaneous death in a cat. This observation of GAEHTGENS has since been confirmed by GLAUSE and LUCHSINGER, BRIEGER, and BOEHM. The chlorid of cholin produces in animals the same muscarin-like symptoms of poisoning as are developed by the vinyl base neurin, the only difference lies in the intensity of the action. In order to bring about a physiological disturbance, cholin must be given in relatively large doses. Thus BRIEGER found it necessary to give about 0.1 gram of cholin chlorid hypodermically to a one-kilogram rabbit in order to bring out the same effects as are obtained by the injection of 0.005 gram of the neurin salt. He also found that the fatal dose for a one-kilogram rabbit was about 0.5 gram, which is about ten times as large as the fatal dose of neurin chlorid. BOEHM observed that doses of 0.025–0.1 gram produced in frogs general paralysis, which, in a short time, leads to death or recovery; and that in its curara-like paralyzing action cholin resembles artificial muscarin, although the latter is about five hundred times stronger. Atropin, as in

the case of neurin and muscarin, antagonizes the action of cholin. Thus, 0.05 gram of the chlorid produced in a frog in one hour diastolic stoppage of the heart. This condition was removed by the injection of 0.001 gram of atropin, the heart-beat rising to the normal in about fourteen minutes; 0.05 gram of cholin chlorid, given subcutaneously to a rabbit (1250 grams) produced salivation, which lasted but a short time, and did not affect the heart-beat and respiration; 0.10 gram was necessary to bring out all the symptoms; 0.05 gram, given to guinea-pigs, had no effect whatever.

BETAIN (OXYNEURIN), $C_5H_{13}NO_3$.—This base has been well known for some time, because of its occurrence in the vegetable kingdom. Thus, it is present in cotton-seed (BOEHM, RITTHAUSEN and WEGER, MAXWELL), where it is about five times as abundant as cholin; in beet-root juice (*Beta vulgaris*, and hence in beet-root molasses (SCHEIBLER, 1866). It occurs also in cattle-turnip and *Lycium barbarum* (HUSEMANN and MARMÉ, SCHÜTTE, SIEBERT); and is found with cholin and another base in vetch-seeds; in peas a base similar to betain exists (SCHULZE). With cholin it occurs in the roots and leaves of *Scopolia atropoides* (SIEBERT). It occurs in the leaves of the potato plant, *Solanum tuberosum*, but not with cholin (SCHÜTTE); in worm-seed (*Artemisia Cina*) in about 0.5 per cent.; with cholin about 0.1 per cent (JAHNS). The two bases are also present in the sprouts of wheat and malt, betain more abundantly (SCHULZE and FRANKFURT). It does not exist in these substances as such, but is formed from a more complex substance by the action of hydrochloric acid or baryta (LIEBREICH). In this respect it resembles cholin, neurin, and probably muscarin. Quite recently, LIPPMANN (1887) has obtained a lecithin-like body from sugar-beet, which, on heating with baryta, gave oleic acid, glycerin, and phosphoric acid (glycerin-phosphoric acid), and betain. Betain, however, does not seem to be a constant constitu-

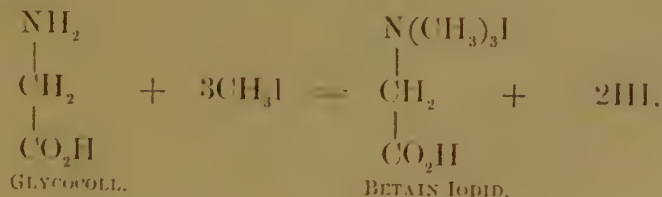
ent, inasmuch as on one occasion he obtained chiefly cholin, and little or no betain. These two bases also occur together in cotton-seed, and this fact has led SCHEIBLER to the conclusion that it is no mere chance. Lecithin, as is well known, may contain variable acid constituents (oleic, stearic, palmitic, etc.), and reasoning on this fact, and on the results of his experiments, LIPPMANN has been led to suppose that it may also contain different bases in variable proportions.

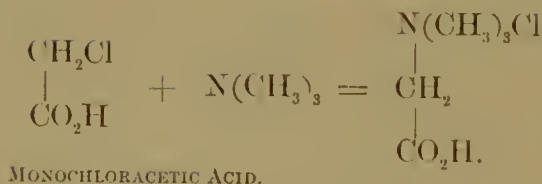
Betain was first discovered by HUSEMANN and MARMÉ in 1863 and 1864 and named *lycine*. SCHEIBLER found it in 1866 in beets, and gave it the present name. The identity of the two compounds was shown in 1875 by HUSEMANN.

A methyl-betain, trigonellin, exists in trigonella (JAHNS, HANTZCH).

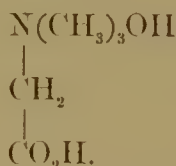
It has been obtained from human urine (LIEBREICH, 1869), and from poisonous and non-poisonous mussel, but not from putrid mussel (BRIEGER, 1855, III., 76). The method for its separation from mussel is described on page 387.

Betain may be obtained synthetically in several ways: (1) by oxidation of cholin with potassium permanganate; (2) by heating sarkosin (methyl glycocoll) with methyl iodid and methyl alcohol, or with methyl iodid alone, when betain-methyl ether also forms (PAULMANN); (3) by the action of silver oxide on betain aldehyde; (4) by the action of methyl iodid on glycocoll (KRAUT); (5) by treating monochloroacetic acid with trimethylamin. The last two methods are of value as indicating the constitution of betain, and the changes which take place can be represented by the equations:

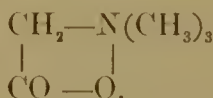




From the formulæ of the salts of betain it is evident that betain has properly the composition $\text{C}_5\text{H}_{13}\text{NO}_3$, which is expressed by the structural formula:



The free base is, however, readily converted into the anhydride, $\text{C}_5\text{H}_{11}\text{NO}_2$, trimethyl glycocoll; the structural formula of which is:



Betain aldehyde was prepared first by BERLINERBLAU and later by FISCHER. On oxidation with silver oxid it yields betain.

Betain is ordinarily regarded as crystallizing with one molecule of water, and the composition is expressed by the formula: $\text{C}_5\text{H}_{11}\text{NO}_2 + \text{H}_2\text{O} (= \text{OH}.\text{N}(\text{CH}_3)_3.\text{CH}_2.\text{CO}_2\text{H})$. It loses this water of crystallization by heating at 100° , or on standing over sulphuric acid, forming an anhydride of the formula already given. LIEBREICH claims that free betain possesses the formula $\text{C}_5\text{H}_{11}\text{NO}_2$, because it yields a compound having the composition $(\text{C}_5\text{H}_{11}\text{NO})\text{ZnCl}_2$. The free base separates from alcohol in large crystals which deliquesce on exposure to the air. As obtained by BRIEGER from the hydrochlorid by treatment with moist silver oxid, it possessed a sweetish taste and neutral reaction. When distilled with potassium hydrate, it yields trimethylamin and other bases, among which a base of the formula $\text{C}_8\text{H}_{17}\text{NO}_5$ occurs in the

largest quantity. In 1893 SCHEIBLER again studied the action of sodium hydrate on betain and found only trimethylamin; no new base, but unchanged betain.

The CHLORID, $C_3H_{12}NO_2.Cl$, forms beautiful crystals, monoclinic plates, which are permanent in the air, and this can be made use of to effect a separation from the cholin salt, which is deliquescent. It crystallizes from aqueous solution in monoclinic plates, from hot saturated 80 per cent. alcoholic solution in beautiful prisms, often several cm. long. It melts at 227° – 228° (JAHNS). It is insoluble in absolute alcohol. This fact can be made use of in their separation (LIPPMANN, MAXWELL). It can, moreover, be easily separated from other bases by its aurochlorid, which is easily soluble. If a little potassio-mercuric iodid is added to a solution of the chlorid, there forms a light-yellow or whitish oily precipitate, which is soluble in excess, but on rubbing the sides of the tube with a glass rod it reappears as yellow needles. This is said to be a characteristic test (BRIEGER, SCHULZE, 1891). By the action of sodium amalgam on aqueous solutions of the chlorid a base is formed, the platinochlorid of which in form, solubility, and composition agrees with muscarin (SCHMIDT, NOTHNAGEL).

The AUROCHLORID, $C_5H_{12}NO_2.Cl.AuCl_3$ ($Au = 43.12$ per cent.), forms magnificent cholesterin-like four-sided plates (or gold yellow needles, PAULMANN), and is easily soluble (BRIEGER). The aurochlorid from sugar-beet is said to crystallize in needles and plates, and to be difficultly soluble in cold water (SCHEIBLER, LIPPMANN). The double salt of the ptomain melts at 209° , and in this it coincides with that obtained from beet-sugar, as well as with that of the synthetically prepared base (BRIEGER). According to SCHÜTTE, it melts at 218° , 220° – 222° ; at 223° – 225° (SIEBERT); at 220° – 221° , decomposing at 222.5° (PAULMANN). The platinochlorid $(C_5H_{11}NO_2.HCl)_2.PtCl_6$ is yellow and crystallizes in prisms. On rapid cooling of hot saturated solution or on precipitation with alcohol it forms more or less anhydrous fine needles; from cold saturated solution over sulphuric acid it crystallizes

in plates which effloresce in the air (JAHNS). It may crystallize with or without water. LIEBREICH obtained crystals with four molecules, while PAULMANN obtained crystals with one molecule of water. JAHNS obtained the salt with three molecules of water.

Betain is not poisonous. It is precipitated with mercuric chlorid together with cholin. SCHULZE and FRANKFURT separate the mercury salts of betain and cholin by partial crystallization; betain is more soluble. The two bases can be separated as chlorids by the solubility in absolute alcohol (MAXWELL, SCHULZE and FRANKFURT, JAHNS).

MUSCARIN, $C_5H_{15}NO_3 = C_5H_{13}NO_2 + H_2O$, the well-known toxic principle which SCHMIEDEBERG and KOPPE obtained from poisonous mushroom (*Agaricus muscarius*), in which it is present accompanied by cholin (HARNACK). BÖHM has found cholin and muscarin together in *Boletus luridus* and *Amanita pantherina*. Later, SCHMIEDEBERG isolated from a commercial specimen of muscarin a base possessing an antagonistic action to muscarin. KOBERT believed that this "fungus-atropin" existed in the fresh mushrooms, and showed that *Russula emetica* contained this compound as well as cholin and muscarin.

This base is especially interesting because of the relation it bears to cholin, for SCHMIEDEBERG and HARNACK showed that it is formed when cholin, or, better still, the platino-chlorid, is oxidized by concentrated nitric acid. NOTHNAGEL by the action of concentrated nitric acid on cholin obtained muscarin, also a nitroso-derivative (nitric acid and cholin ether), and a substance which is the chief product, besides a little muscarin and the nitroso-compound, when the oxidation is carried out with dilute nitric acid. The muscarin from cholin does not combine with phenyl hydrazin; betain aldehyde does. The chlorid on treatment with acetic anhydride or benzoyl chlorid yields an anhydride of muscarin (NOTHNAGEL), the exact composition of which is yet undetermined; the group $—CH \begin{smallmatrix} \nearrow OH \\ \searrow OH \end{smallmatrix}$ in muscarin is

changed either into an aldehyde —COH group, or into $\text{—(CHOH)}_2\text{O}$.

In the preparations of muscarin from cholin a small quantity of a nitroso-compound forms, the platinum salt of which resembles that of muscarin in solubility, but never in form, which is always plumose. These crystals are permanent in the air, and contain two molecules of water which are not driven off at 100° . They melt at $223^\circ\text{--}224^\circ$ with decomposition. It possesses the formula :



The gold salt forms fine, light-yellow needles which are anhydrous and melt at 240° . It gives Liebermann's nitroso reaction—blue color with phenol and sulphuric acid.

A third "muscarin," $\text{OH.N}(\text{CH}_3)_3.\text{CH}_2.\text{COH}$, was prepared by BERLINERBLAU by the action of baryta on trimethylamin and chloroacetal. FISCHER prepared the same compound by the action of concentrated hydrochloric acid on acetal-trimethyl ammonium hydroxide. This base, however, differs from muscarin by the elements of water—anhydro-muscarin. In reality it is betain aldehyde, since on oxidation with moist silver oxide it yields betain (FISCHER). Unlike real muscarin, it has no action on the heart of frogs or on the pupils of birds (MEYER). Like most ammonium bases it induces strong salivation and perspiration. SCHMIEDEBERG found it to resemble cholin in its action, whereas LUCHSINGER found it to agree in its action with muscarin.

A fourth base, oxycholin $\text{—OH.N}(\text{CH}_3)_3.\text{CH.OH.CH}_2\text{OH}$, was prepared by BODE by the action of silver oxide on hypochlorous acid and neurin chlorid. Its platinum salt melts at 254° (NOTHAGEL). Its physiological action is different from that of muscarin. Thus, in frogs it slows the heart, but does not cause stoppage. Atropin counteracts its action. In mammals the pulse is lowered as a result of stimulation of the central vagus ganglia, 10–15 mg. intravenously stimulate the intracardiac exhibition. The blood-pressure is not lowered as is the case in muscarin, but is somewhat raised. Neither

the intestines nor the iris of mammals is affected. The iris of birds is contracted as with muscarin, and the glands are affected. In cats and guinea-pigs salivation and flow of tears result. Like all ammonium bases it has a marked curara-action.

A fifth base, resembling fungus- and cholin-muscarin in the form, solubility, and composition of the platinochlorid, was obtained by the action of sodium amalgam on aqueous solutions of betain chlorid (SCHMIDT).

Lastly, BRIEGER in 1885 (I., 48) isolated a muscarin base from haddock which had been allowed to decompose for five days. The process by which its isolation was effected is described on page 391. GULEWITSCH isolated a small amount of a substance resembling muscarin, together with cholin and cadaverin, from horseflesh kept at $15^{\circ} +$ for four months.

It is barely possible that BRIEGER's base is distinct from SCHMIEDEBERG's; nevertheless, it closely resembles it and apparently is identical.

The CHLORID, $C_5H_{14}NO_2.Cl$, is obtained on the decomposition of the platinochlorid with hydrogen sulphid, as a syrupy residue, which, under the desiccator, shows a tendency to crystallize gradually (BRIEGER). It is deliquescent (HARNACK). A commercial muscarin sulphate was found to be chiefly cholin (NOTHNAGEL).

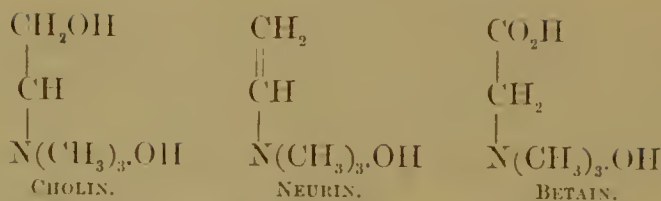
The PLATINOCHLORID, $(C_5H_{14}NO_2.Cl)_2PtCl_4$ (Pt = 30.08 per cent., BRIEGER), forms as a crystalline deposit of pin-head size, more or less well formed octahedra, which are difficultly soluble in water. They lose their water of crystallization ($2H_2O$) only by means of strong heating (BRIEGER, NOTHNAGEL). It melts at about 240° with decomposition.

The AUROCHLORID, $C_5H_{14}NO_2.Cl.AuCl_3$ (Au = 42.82 per cent.), crystallizes in needles, and is difficultly soluble in water (BRIEGER); more difficultly soluble than the cholin double salt (HARNACK). From hot hydrochloric acid water it crystallizes as light-yellow, glistening platelets (NOTHNAGEL). It is scarcely to be distinguished from the corresponding salt of cholin (NOTHNAGEL). It begins to run together at 174° ,

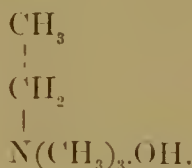
gradually melting, and decomposes at 232° . It has no water of crystallization. The separation of muscarin and cholin is very difficult. HARNACK separated the two by spreading the mixed chlorids on a filter-paper which absorbed the muscarin salt. NOTHNAGEL separated the two bases as platinochlorid by repeated recrystallization from hot water and washing the crystals with cold water. The platinochlorid of cholin is easily soluble in water.

Physiological Action.—Small doses of this ptomain induce in frogs total paralysis, with stoppage of the heart in diastole, and this action is antagonized by subsequent injection of atropin, but in the case of previously atropinized frogs it fails to antagonize. Very small doses produce in rabbits profuse salivation and lachrymation, contraction of the pupil, profuse diarrhoea, and passage of urine and semen; finally, the animal dies in convulsions, which, however, are only of short duration (BRIEGER). Although the natural and artificial muscarin and their salts are chemically and physically alike, they are not, however identical, although so considered by SCHMIEDEBERG and HARNACK. This is seen in their physiological action. Thus BÖHM found that the artificial muscarin paralyzed intramuscular nerve-endings. According to MEYER, $\frac{1}{10}$ — $\frac{1}{20}$ mg. will do this, whereas the natural base will not have this effect. Again, 1–2 drops of a 1 per cent. solution of the artificial base will produce maximal myosis in birds in a few minutes; while the natural base has no effect on birds' pupils. The action of betain aldehyde and iso-muscarin has already been stated. BRIEGER'S ptomain would seem to be nearly identical with the artificial muscarin.

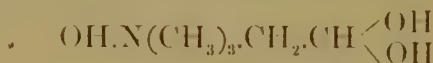
CONSTITUTION OF THE MEMBERS OF THE CHOLIN GROUP.
—The structure of cholin was clearly demonstrated by WURTZ, who accomplished the synthesis of this base by treatment of ethylene chlorhydrin with trimethylamin. This same method can be applied to the synthesis of betain and neurin by using monochloroacetic acid and vinylbromid instead of ethylene chlorhydrin. The structural formulae which can be deduced from these reactions are as follows:



All these bases, since they can be prepared from cholin, may also be considered as oxidation-products of trimethyl-ethyl-ammonium hydrate :



The constitution of muscarin is still unsettled. SCHMIEDEBERG and HARNACK believed that it resulted from cholin by the oxidation of hydrogen connected with the same carbon as the hydroxyl group. Its formula would be either

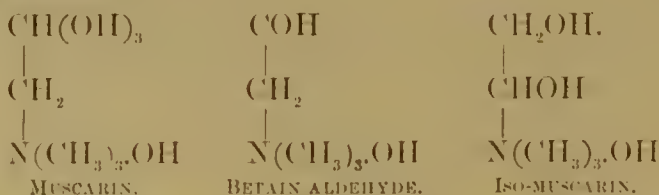


or



The former, analogous to chloral, would have two hydroxyl groups attached to one carbon atom. The presence of hydroxyl groups in muscarin cannot, however, be demonstrated with acetic anhydrid, or benzoyl chlorid (NOTHLAGEL). The second formula is that of betain aldehyd.

The formulæ of the several muscarin compounds are here-with presented :



It will be observed that very slight differences in the chemical constitution of cholin, muscarin, betain, and neurine are accompanied by very great differences in the physiological

action of these bases. Thus, as pointed out by SCHMIDT, cholin may be considered as a primary alcohol and betain as a monobasic acid. Between these two relatively non poisonous bases is the intermediate oxidation-product, the aldehyde muscarin, which is highly poisonous.

Again, it will be remembered that the artificial muscarin formed by the oxidation of cholin had a markedly different physiological effect on the intramuscular nerve-endings and on the pupils of birds from that of the natural muscarin. This difference must undoubtedly be ascribed to difference in stereochemical structure, as in the case of active and inactive lactic acids, of atropin. Furthermore, BONE's iso-muscarin possesses likewise an entirely different action, differing only in the position of the hydroxyl group. The same is true of betain aldehyde, which differs from muscarin by the elements of water.

The relatively non-poisonous cholin is readily converted into the poisonous neurin, or, *vice versa*, neurin may be changed into cholin by removing or respectively adding the elements of water.

MYDATOXIN, $C_6H_{13}NO_2$. — This base was obtained by BRIEGER in 1886 (III., 25, 32) from several hundred pounds of human internal organs which were allowed to stand in closed but spacious wooden barrels for four months, at a temperature varying from -9° to $+5^\circ$. He obtained much larger quantities of it, however, from horseflesh which had putrefied under the same conditions. In the process of extraction it is found in the mercuric-chlorid precipitate together with cadaverin, putrescin, and another base, $C_7H_{17}NO_2$. It can be isolated from this mixture by recrystallizing the mercury salts, which removes the cadaverin, because of its difficult solubility in water, and decomposing the soluble mercury salts by hydrogen sulphid. The filtrate freed from mercury is now evaporated to dryness and the residue repeatedly extracted with absolute alcohol, in order to remove the putrescin hydrochlorid, which is insoluble. The alcoholic solution, after standing some time to permit complete separation of any dis-

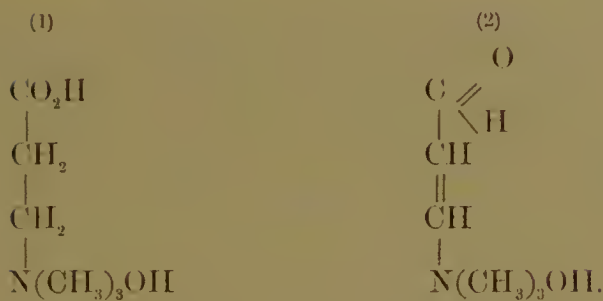
solved putrescin, is then evaporated to dryness and taken up with water. This solution gives, on the addition of gold chlorid, a precipitate of the aurochlorid of the base $C_7H_{17}NO_2$. The filtrate from this precipitate, containing the mydatoxin, is treated with hydrogen sulphid to remove the gold, and then evaporated to dryness. The colorless, syrupy hydrochlorid thus obtained forms with platinum chlorid a double salt which is readily soluble in water, and can be purified by repeated recrystallizations from absolute alcohol containing some hydrochloric acid.

The name mydatoxin is derived from *μῡδαίνω*, to putrefy. The free base is obtained from the hydrochlorid by treatment with moist, freshly precipitated silver oxid, as a strongly alkaline syrup, which solidifies in vacuo to plates. It is insoluble in alcohol, ether, etc. It does not distil without decomposition. It is isomeric with the base $C_6H_{13}NO_2$, obtained by BRIEGER in 1888 from tetanus-cultures.

The HYDROCHLORID, $C_6H_{13}NO_2 \cdot HCl$, is a colorless deliquescent syrup which does not form any double salt with gold chlorid. With platinum chlorid it gives an easily soluble salt. Otherwise it combines only with phosphomolybdic acid, with which it forms cubes. Ferric chlorid and potassium ferrieyanid yield, after a time, Berlin-blue. It is readily soluble in alcohol.

The PLATINOCHLORID, $(C_6H_{13}NO_2 \cdot HCl)_2PtCl_4$ (Pt = 29.00 per cent.), melts at 193° , with decomposition. It crystallizes in plates which are extremely soluble in water. It can be readily recrystallized from absolute alcohol acidulated with hydrochloric acid. The mercury salt is readily soluble in water.

The exact formula of this base, of mytilotoxin, and some other bases, cannot be considered to be permanently settled, inasmuch as the formula of the hydrochlorid, $C_6H_{13}NO_2 \cdot HCl$, as deduced from the analysis of the platinum double salt, may equally apply to the base $C_6H_{11}NO_2OH$ as to the base $C_6H_{11}NO_2$. If the first formula is correct, then mydatoxin is a homologue of betain, and its structure would be expressed by (1).



The second formula would seem to correspond to an unsaturated aldehyde of the cholin group, and its structure may be indicated by (2).

This ptomain, although it possesses toxic properties, is not, however, a strong poison. Its action is the same as that of the base $\text{C}_7\text{H}_{17}\text{NO}_2$ (see page 395), with which it is associated, except that the symptoms of poisoning develop slower, so that the death of a guinea-pig does not take place for about twelve hours. White mice are very susceptible to the action of these two poisons. A short time after the injection of even small doses they are taken with convulsions which come on in paroxysms. The eyeballs roll upward. Lachrymation, diarrhœa, and dyspnœa come on, and the mice die within a short time.

A BASE(?), $\text{C}_6\text{H}_{13}\text{NO}_2$, an isomer of the preceding, was obtained by BRIEGER in 1888 from tetanus-cultures. It is not poisonous—distinction from mydatoxin. It probably is an amido-acid. The platinochlorid crystallizes in plates, is easily soluble in water and in alcohol, and melts at 197° with decomposition (see page 400).

MYTILOTOXIN, $\text{C}_6\text{H}_{13}\text{NO}_2$, is the specific poison of toxic mussel (*Mytilus edulis*), from which it was obtained by BRIEGER in 1885 (III., 76). This poison is formed during the life of the animal under certain conditions which have been thoroughly studied by SCHMIDTMANN, VIRCHOW, and others (see p. 45). BRIEGER obtained the poison by extracting toxic mussel with acidulated water, and evaporating

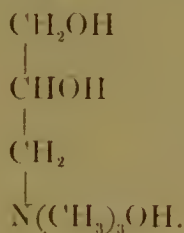
this solution to a syrupy consistency. The residue was thoroughly extracted with alcohol, and this solution was treated with lead acetate, in order to remove mucilaginous substances. The filtrate was then evaporated, and the residue extracted with alcohol. Any lead that had dissolved was removed by hydrogen sulphide. The alcohol was expelled, and the resulting syrup was taken up with water and decolorized by boiling with animal charcoal. The clear solution was now neutralized with sodium carbonate, acidulated with nitric acid, and precipitated with phosphomolybdic acid. The precipitate was decomposed by warming with neutral lead acetate, and the resulting filtrate, after the removal of the lead by hydrogen sulphide, was acidulated with hydrochloric acid and evaporated to dryness. The residue was extracted with absolute alcohol, whereby betain, on account of its insolubility, is removed, and the alcoholic solution was precipitated by alcoholic mercuric chlorid. The mercury precipitate is repeatedly recrystallized from water, and the poison is obtained as an easily soluble double salt.

The free base as obtained by the addition of alkali to the hydrochlorid possesses a disagreeable odor which disappears on exposure to air, and the substance ceases to possess poisonous properties. BRIEGER has proposed the application of this test for the recognition of poisonous mussel; on treatment of these with alkali the characteristic odor is developed. Mytilotoxin is also destroyed on distillation with potassium hydrate, and in the distillate there are found an aromatic non-poisonous product and trimethylamin. The free base, therefore, does not exist by itself for any length of time, but soon becomes converted into an inert substance. H. SAL-KOWSKI has also shown that it is destroyed on boiling with potassium carbonate, whereas its hydrochloric acid solution can be evaporated to dryness, and heated to 110° without destroying its poisonous property.

The HYDROCHLORID, $C_6H_5NO_2.HCl$, prepared from the aurochlorid, crystallizes in tetrahedra. It is extremely poisonous, and according to BRIEGER produces exactly the same

symptoms which have been observed by SCHMIDTMANN in persons who have partaken of poisonous mussels (see page 44). On standing, however, the pure hydrochlorid gradually becomes dark, and decomposes with loss of its poisonous property—a change corresponding to that which tetanin undergoes (p. 400). The gold salt is better adapted for preservation. The ordinary alkaloidal reagents produce in its solutions, if at all, only oily precipitates.

As stated under mydatoxin, the formula of the hydrochlorid, $C_6H_{15}NO_2.HCl$, is applicable to either one of the two bases, $C_6H_{16}NO_2.OH$ or $C_6H_{15}NO_2$. The base corresponding to the first formula is evidently a homologue of muscarin, and should possess a similar physiological action. As a matter of fact, mytilotoxin does resemble muscarin somewhat in its action, and its occurrence together with betain would seem to make it a decomposition-product of lecithin, in which case this base must be looked upon as a member of the cholin group. It is interesting to know that a compound corresponding to the formula $C_6H_{16}NO_2.OH$ has been known for some time, and was prepared by HANRIOT in a manner analogous to WURTZ's synthesis of cholin, by treating glycerin mono-chlorhydrin with trimethylamin. This base, trimethyl-glyceryl-ammonium hydrate, has this structure :



It would seem that HANRIOT's base might possibly be identical with mytilotoxin, but a careful comparison made by BRIEGER showed that it possesses no physiological action, and that its chemical reactions are entirely different.

Mytilotoxin would, therefore, seem to possess the formula $C_6H_{15}NO_2$, as originally given by BRIEGER. From the fact that on distillation with potassium hydrate it yields trimethyl-

amin, it follows that mytilotoxin is a quarternary base. He is inclined to regard it as a methyl derivative of betain, which is so common in mussels, and represents it by formula No. 1.



No. 1, however, is $\text{C}_6\text{H}_{15}\text{NO}_3$, instead of $\text{C}_6\text{H}_{15}\text{NO}_2$, as above. The formula No. 2, $\text{C}_6\text{H}_{17}\text{NO}_2$, would represent a derivative of cholin or muscarin, with only a slightly higher percentage of hydrogen.

The AUROCHLORID, $\text{C}_6\text{H}_{15}\text{NO}_2.\text{HCl}.\text{AuCl}_3$ ($\text{Au} = 41.66$ per cent.), crystallizes in cubes. Its melting-point is 182° .

It is well to observe that BRIEGER has been unable to obtain this base from mussels that were allowed to putrefy for sixteen days.

Physiological Action.—According to BRIEGER, mytilotoxin produces all the characteristic effects seen in mussel poisoning, and it is, therefore, a strong paralysis-producing poison, and resembles curara in its action. This action is explainable now that GLAUSE and LUCHSINGER have shown that all trimethyl ammonium bases have a muscarin-like action. For the symptoms induced by poisonous mussel see page 44.

GADININ, $\text{C}_7\text{H}_{17}\text{NO}_2$, was found in haddock (1885) which was allowed to decompose in open iron vessels for five days during summer. BRIEGER has also obtained it from cultures of the bacteria of human feces on gelatin. CARBONE has found it in cultures of the *Proteus vulgaris*. The decomposing mass was thoroughly stirred every day in order to bring it into contact with atmospheric oxygen (BRIEGER, l., 49). It was then treated with water, and hydrochloric acid was added to acid reaction, and after being warmed the mixture was filtered and the filtrate concentrated on the water-bath to a syrupy consistency. This syrupy residue was extracted with water, and the aqueous solution was precipitated with a solution of

mercuric chlorid. This mercuric-chlorid precipitate contained a base, the quantity of which, however, was insufficient for a complete analysis (see page 405). The mercuric-chlorid filtrate, after the removal of the mercury by hydrogen sulphid, was evaporated to a syrup, and this was then repeatedly extracted with alcohol. The alcoholic solution thus obtained contained neuridin, a base of the same composition as ethylenediamin, muscarin, gadinin, and triethylamin. These bases were separated in the following manner: The alcoholic solution gave with platinum chlorid a precipitate of neuridin. The filtrate from this platinum precipitate was heated on the water-bath to expel the alcohol, and then the platinum was removed by hydrogen sulphid. The aqueous filtrate was concentrated to a small volume which, on addition of platinum chlorid, gave a precipitate of the isomer of ethylenediamin. The mother-liquor from this precipitate was concentrated on a water-bath, and on cooling the platinochlorid of muscarin crystallized out. From the mother-liquor of this precipitate on standing in a desiccator, the gadinin double salt crystallized. The mother-liquor from the gadinin platinochlorid was treated with hydrogen sulphid to remove the platinum, and the aqueous filtrate on distillation with potassium hydrate gave triethylamin.

Gadinin (from *Gadus callarias*, haddock) in small doses does not appear to be poisonous; large doses (0.5–1 gram) are decidedly toxic and may kill guinea-pigs. The formula of the free base as deduced from the analysis of the platinochlorid may be either $C_7H_{17}NO_2$ or $C_7H_{18}NO_2.OH$.

The HYDROCHLORID, $C_7H_{17}NO_2.HCl$, as obtained by the decomposition of the platinochlorid with hydrogen sulphid, crystallizes under the desiccator in thick, colorless needles, which are easily soluble in water; insoluble in alcohol. It forms no combination with gold chlorid, but does give crystalline precipitates with phosphomolybdic acid, phosphotungstic acid, and picric acid.

The PLATINOCHLORID, $(C_7H_{17}NO_2.HCl)_2PtCl_4$ ($Pt = 27.68$ per cent.), is at first quite soluble, and on standing over a

desiccator it crystallizes in golden-yellow plates, which, when once formed, are again difficultly soluble in water. It can be recrystallized from hot water. It melts at 214° .

TYPHOTOXIN, $C_7H_{17}NO_2$.—This base was named thus by BRIEGER in 1885 (III., 86), and was regarded by him as the specific toxic product of the activity of Koch-Eberth's typhoid bacillus. It is, however, probable that, as in the case of tetanus, there are basic and other products formed. He obtained it by cultivating the bacillus on beef-broth for eight to fourteen days at the temperature 37.5 – 38° . The nature of the soil on which it grows has a great deal to do with the formation of the poison. An especially important factor is the temperature: for BRIEGER has observed that no poison was produced in one case where the temperature remained by accident at 39° for twenty-four hours. In such cases creatin is present in quantity, whereas otherwise the reverse is the rule.

In the process of extraction it occurs in the mercuric chlorid precipitate, and from this it is obtained, after the removal of the mercury by hydrogen sulphid, as an easily deliquescent hydrochlorid. This for the purpose of purification is converted into the difficultly soluble anrochlorid.

Typhotoxin is isomeric with gadinin and the compound $C_7H_{17}NO_2$, which BRIEGER obtained from putrefying horse-flesh. In its properties it is, however, very different. Thus, the free base is strongly alkaline, and its hydrochlorid yields a difficultly soluble picrate. On the other hand, the isomer from horseflesh possesses a slightly acid reaction, and does not form a picrate. Again, typhotoxin gives with Ehrlich's reagent (sulpho-diazobenzole) an immediate yellow color, which disappears upon the addition of alkali, whereas the isomer does not give this reaction. Furthermore, the two bases differ in their physiological action and in their behavior to alkaloidal reagents (see Table I.). Their anrochlorids, however, possess the same melting-point.

The HYDROCHLORID is readily deliquescent, and unites

with platinum chlorid to form an easily soluble double salt crystallizing in needles.

The AUROCHLORID, $C_7H_{17}NO_2.HCl.AuCl_3$ (Au = 40.46 per cent.), is difficultly soluble, and crystallizes in prisms, which melt at 176° . In its melting-point and solubility (197° , BRIEGER, *Arch. f. pathol. Anat.*, **115**, 489) it agrees with its isomer from horseflesh. From some of his first experiments in the cultivation of the typhoid bacillus, BRIEGER (II., 69) obtained a basic product differing in some of its characters from typhotoxin. Its aurochlorid, on analysis, gave 41.91 and 41.97 per cent. of Au, 16.06 per cent. of C, and 3.66 per cent. of H.; while typhotoxin aurochlorid gave 40.78 per cent. Au, 17.38 per cent. C, and 3.85 per cent. H. For a comparison of the reaction of these two substances, see Table I.

In its physiological action typhotoxin differs from its isomer (page 395) in that the latter produces symptoms with well-marked convulsions, whilst the former throws the animal into more of a paralytic or lethargic condition. The action of this base has been studied only on mice and guinea-pigs. It produces at first slight salivation with increased respiration; the animals lose control over the muscles of the trunk and extremities, and fall down helpless upon their sides. The pupils become strongly dilated, and cease to react to light; the salivation becomes more profuse; the rate of heart-beat and of respiration gradually decreases, and death follows in from one to two days. Throughout the course of these symptoms the animals have frequent diarrhetic evacuations, but at no time are convulsions present. On post-mortem the heart is found to be in systole, the lungs are strongly hyperemic, the other internal organs pale, the intestines firmly contracted, and their walls pale.

A BASE(?), $C_7H_{17}NO_2$, was obtained by BRIEGER in 1886 (III., 28) on working over about one hundred pounds of horseflesh which had been allowed to undergo slow putrefaction with limited access of air and at a low temperature (-9° to $+5^\circ$) for four months. It occurs in the mercuric-

chlorid precipitate together with cadaverin, putrescin, and tydatoxin, and from these bases it can be separated and isolated according to the method on page 385.

A similar, if not identical, substance, having the composition $C_7H_{17}NO_2$, was obtained by BAGINSKY and STADTHAGEN (1890) from cultures on horseflesh, ten days at 35° , of a bacillus, closely allied to Finkler-Prior's, and isolated from stools of cholera infantum. The gold salt in crystalline form and properties is the same as BRIEGER's, except that it possesses a somewhat higher melting-point.

The free substance possesses, even after most careful purification, a slightly acid reaction. This acidity is removed from even a large quantity of the substance by the addition of a drop of alkali. On account of the acid character of the free substance BRIEGER does not consider it to be a base (a ptomain). It differs, however, from the amido-acids in its poisonous character; in the fact that, unlike an acid, it does not unite with bases to form salts; and in not giving the characteristic red coloration (Hofmeister's reaction for the amido-acids) with ferric chlorid. Whatever the true nature of this substance may be, it nevertheless, in its other properties, behaves like a base. Thus, it forms simple as well as double salts. On boiling with copper acetate it gives amorphous floccules. Under the desiccator it solidifies into plates which deliquesce on exposure to the air. It does not combine either with silver oxide or with cupric hydrate. On dry distillation it yields a distillate possessing a strong acid reaction and a peculiar odor. The distillate does not give any precipitate with platinum chlorid, or with gold chlorid, nor does it react with copper acetate. With phosphomolybdic acid, however, it forms an amorphous mass; with ferric chlorid and potassium ferricyanid it yields an immediate precipitate of Berlin-blue, whereas the original substance does not give any blue coloration.

The HYDROCHLORID, $C_7H_{17}NO_2.HCl$, crystallizes in fine needles which are insoluble in absolute alcohol. When its aqueous solution is treated with freshly precipitated silver oxid

the resulting filtrate contains some silver oxid in solution, from which it can be removed by hydrogen sulphid; thus differing from an ammoniacal silver solution, which gives no precipitate on treatment with hydrogen sulphid. In this respect it resembles SALKOWSKI'S base, page 354. For reactions of the hydrochlorid, see Table I.

The AUROCHLORID, $C_7H_{17}NO_2.HCl.AuCl_3$, forms plates which are difficultly soluble in water, and melt at 176° —the melting-point of the gold salt of typhotoxin. It is dimorphous, since sometimes it is also obtained in needles which can be changed into plates.

It does not form a picrate, nor does it give a reaction with sulpho-diazobenzole.

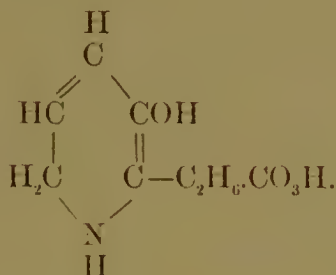
Physiological Action.—This substance, when injected into frogs, produces a curara-like action. A few minutes after the injection the animal falls into a condition of paralysis, and, although it can still react toward reflexes, it cannot move from its place. At times fibrillary twitchings pass over the body. The pupils dilate, the heart-action becomes gradually weaker, and finally, after several hours, the animal dies, with the heart in diastole. Doses of 0.05 to 0.3 gram of the hydrochlorid, injected into guinea-pigs, produce in a short time a slight tremor, gradual increase in respiration, and slight moistening of the lower lip. The pupils at first contract, then dilate *ad maximum*, and become reactionless. The temperature remains at first normal; chills of short duration follow in rapid succession. The animal squats on the ground, with its snout pressing against the floor in exactly similar manner as is caused by the mussel-poison. Violent clonic convulsions follow in continually shorter intervals, and at the same time lachrymation and salivation become profuse, but not so excessive as in the case of the muscarin-like ptomaines. The temperature sinks with the decrease in the rate of respiration, the ears previously gorged become pale and cold, and the heart-action becomes irregular and less frequent than before. General paralysis sets in, but the head still moves upward and backward. External stimuli induce violent clonic convulsions, the

animal repeats frequently choking movements, and at the same time yields large quantities of saliva; finally, it falls upon its side completely paralyzed, and dies. The heart stops in diastole, the intestines are pale and strongly contracted, and the bladder is empty and likewise contracted.

MORRHUIC ACID, $C_9H_{13}NO_3$, was obtained by GAUTIER and MOURGUES (1888) from brown cod-liver oil, together with six bases already described—namely, butylamin, amylamin, hexylamin, dihydrolutidin, asellin, and morrhuin. These bases constitute about 0.2 per cent. of the oil. The discoverers regard them as true leucomains, dissolved from the hepatic cells by the oil. BOUILLOR found that the mixed bases, or total basic product, in a dose of 0.25–0.15 g. in man increased the volume of urine and the quantity of urea. By a micro-chemical reaction, exposing sections of liver to the fumes of hydrochloric or hydrofluoric acid, he detects the bases in the liver, especially in the bile-ducts. The bases, therefore, exist preformed in the cod's liver, and are derived from the bile. It is more probable, however, that these compounds are the products of initial decomposition, and for that reason they are described under the head of ptomaines. This compound is relatively abundant, and is basic as well as acid in character. It is resinous in appearance, and can be crystallized in flattened prisms, or large lance-shaped plates. When recently precipitated it is oleaginous, viscons, then gradually hardens. It possesses a disagreeable aromatic odor resembling that of the sea-weeds upon which the fish feed. According to the discoverers, its probable source is the lecithin derived thus from these weeds. It is soluble in alcohol, and but slightly in ether. It reddens turmeric, decomposes carbonates, and with acids forms salts which precipitate lead acetate and silver nitrate, but not copper acetate, even on warming.

The hydrochlorid is crystalline, and is partially dissociated by excess of water. The platinum salt is soluble, and crystallizes in very small cross-shaped prismatic needles. The gold salt is amorphous, and is readily altered on heating.

The properties of this compound show that it is of a pyridin nature, and inasmuch as it does not give a precipitate with copper acetate, it would appear that the carboxyl is not directly united to the pyridin-nucleus. This does not necessarily follow, now that we know that some amido-acids exist which do not give a reaction with copper acetate (see page 354). Its pyridin nature is futhermore shown on distillation with lime. An oily alkaline base is thus obtained which forms an iodomethylate, and this with potassium hydrate yields quite an intense red color, resembling lees (De Coninck's reaction). On oxidation with permanganate of potassium it yields a mono-basic acid. According to GAUTIER and MOURGUES, the compound is probably identical with DE JUNGH's gaduin, and they ascribe to it the following constitution, which, it should be said, lacks full confirmation :



Compare with tyrosin, $\text{C}_9\text{H}_{11}\text{NO}_3$ (page 313).

A BASE, $\text{C}_5\text{H}_{12}\text{N}_2\text{O}_4$, was obtained by POUCHET (1884) from the residual liquors resulting from an industrial treatment of débris of bones, flesh, and waste of all kinds, with dilute sulphuric acid. It is accompanied by another base, $\text{C}_7\text{H}_{18}\text{N}_2\text{O}_6$, from which it can be separated by treatment with alcohol. The base itself forms tufts of delicate needles which alter or decompose less easily than the accompanying base. The platinochlorid, $(\text{C}_5\text{H}_{12}\text{N}_2\text{O}_4.\text{HCl})_2\text{PtCl}_6$, forms a dull yellow powder, somewhat soluble in strong alcohol, but insoluble in ether. The platinochlorid, $(\text{C}_7\text{H}_{18}\text{N}_2\text{O}_6.\text{HCl})_2\text{PtCl}_6$, is insoluble in ether.

The hydrochlorids of these bases form silky needles, which

are altered by excess of hydrochloric acid and by exposure to air. *POUCHET* considers them to be closely allied to the oxybetains. The general alkaloidal reagents precipitate these bases; the phosphomolybdic precipitate, on addition of ammonia, gives a blue tint. Both bases are toxic, and exert a paralyzing action upon the reflex movements.

The method employed by *POUCHET* for their isolation was to precipitate them as tannates. The precipitate was decomposed by lead hydrate in the presence of strong alcohol, the excess of lead removed from the solution by hydrogen sulphide, and the clear liquid thus obtained was submitted to dialysis. The above bases occurred in the dialysate. In the non-dialyzable portion volatile bases were found probably identical with those described by *GAUTIER* and *ETARD*.

TETANIN, $C_{13}H_{30}N_2O_4$, was obtained in 1886 by *BRIEGER* (III., 94) by cultivating impure tetanus-microbes of *Rosenbach*, in an atmosphere of hydrogen on beef-broth for eight days at 37° – 38° . It likewise occurs in cultures on brain-broth. Later (April, 1888), *BRIEGER* succeeded in obtaining tetanin from the amputated arm of a tetanus-patient, identical in its physiological action and chemical reactions with that isolated from cultures of *Rosenbach's* germs on beef-broth. The presence of tetanin during life in tetanus-patients has thus been demonstrated. It has not been found in the brain and nerve tissue of persons dead from tetanus. A portion of the jelly-like mass taken from the amputated arm was found to contain tetanus-bacilli as well as staphylococci and streptococci, and when planted on beef-broth tetanin was formed, but no tetanotoxin or spasmotoxin.

KITASATO and *WEYL* (1890), employing pure cultures of the tetanus-bacillus, obtained from 1½ kilogram beef used as culture-medium 1.7118 gram of tetanin hydrochlorid (0.137 per cent.). Tetanotoxin was also present.

For its isolation *BRIEGER* employed the following method: The cultures were slightly acidulated with hydrochloric acid, heated and filtered; the filtrate was then treated with lead

acetate and with alcoholic mercuric chlorid in the manner described under mytilotoxin (page 388). KITASATO and WEYL digest the cultures with 0.25 per cent. hydrochloric acid for some hours at 60° , then render slightly alkaline, filter, and distil in vacuo at 60° . The residue in the retort is worked for tetanin by Brieger's method, while the distillate contains tetanotoxin, ammonia, indoI, hydrogen sulphid, phenol, and butyric acid. The filtrate from the above mercuric-chlorid precipitate contains the greater part of the active principle, provided the precipitate has been thoroughly washed. After the removal of the mercury by hydrogen sulphid it is evaporated, and the residue is repeatedly extracted with absolute alcohol, in which the tetanus-poison readily dissolves, and can thus be separated from the insoluble ammonium chlorid. The alcoholic solution is treated with alcoholic platinum chlorid, which precipitates the ammonium and creatinin platinochlorids, whilst the platinochlorid of the poison remains in solution. The filtrate from this precipitate gives, on the addition of ether, a flocculent precipitate possessing exceedingly deliquescent properties. The precipitate is, therefore, rapidly filtered off by means of a pump, and dried in vacuo. It can then be recrystallized from hot 96 per cent. alcohol, and the beautiful clear-yellow plates thus obtained, if dried again in vacuo, become rather difficultly soluble in water, from which it can then be recrystallized and obtained in a perfectly pure condition. If boiled with boneblack, it decomposes, yielding a non-poisonous crystalline compound.

Phosphomolybdic acid cannot be used in the separation of tetanin, inasmuch as it destroys the poison (BRIEGER). BOCKLISCH has also observed that it destroys the poison formed in the putrefaction of fish.

Tetanin obtained by treating the hydrochlorid with freshly precipitated moist silver oxide forms a strongly alkaline yellow syrup. With alkaloidal reagents it gives the same reactions as the hydrochlorid, except that it does not give a blue color with ferric chlorid and potassium ferrieyanid. It is easily decomposed in acid solution, but is permanent in alkaline solution.

The HYDROCHLORID, $C_{13}H_{30}N_2O_4 \cdot 2HCl$, is very deliquescent, and is easily soluble in absolute alcohol. Besides with platinum it combines only with phosphomolybdic acid to form an easily soluble crystalline precipitate, which on the addition of ammonium hydrate becomes white. If, however, the hydrochlorid is impure, phosphomolybdic acid produces a precipitate which is colored an intense blue by ammonia. Potassium-bismuth iodid yields a precipitate which is at first amorphous, but soon becomes crystalline. Ferric chlorid and potassium ferrieyanid produce a slowly developing blue color, which probably is due to impurities.

When kept for some months the highly poisonous hydrochlorid becomes syrupy, brownish, and wholly inert. Examined at this stage, the syrup was found, by means of platinum chlorid, to contain a substance the hydrochlorid of which crystallized in plates. This is readily soluble in water and alcohol, and melts at 197° with total decomposition, the same as tetanin. It combines only with phosphomolybdic acid to form an easily soluble compound. The platinum salt has the composition $C_6H_{13}NO_2 \cdot 2HCl \cdot PtCl_4$. This substance is non-poisonous, and probably an amido-acid. It is different, however, from leucin and Nencki's isomers of leucin, although possessing the same composition. It is also isomeric with mydatoxin, $C_6H_{13}NO_2$, but this is highly poisonous to mice, while the former is inert (see page 387). Tetanin resembles mytilotoxin with respect to this loss of toxicity on standing.

The PLATINOCHLORID, $C_{13}H_{30}N_2O_4 \cdot 2HCl \cdot PtCl_4$ (Pt = 28.33 per cent.), is easily soluble in absolute alcohol, from which it is precipitated on the addition of ether. From ninety-six per cent. alcohol it crystallizes in clear yellow plates. After repeated recrystallization from alcohol and drying in vacuo it becomes difficultly soluble in water so that it can be recrystallized from the latter. It decomposes at 197° .

This base produces the characteristic, though probably not all the symptoms of tetanus, since we know of at least three other toxins (pages 172, 310, 311) which occur with tetanin in cultures of the tetanus-microbe. The symptoms induced

by relatively large doses in warm-blooded animals, as mice, guinea-pigs, and rabbits, exhibit two distinct phases. In the first, the animal is thrown into a lethargic, paralytic condition, then suddenly becomes uneasy, and the respiration becomes more frequent. This is followed by the second phase, in which tonic and clonic convulsions, especially the former, predominate till death results. 0.5 gram has but slight action on guinea-pigs. Small doses do not seem to affect guinea-pigs, while frogs seem to be much less sensitive than mice. The characteristic convulsions and opisthotonus seen in tetanus in man are also produced in guinea-pigs on injection of large doses of this base. Dogs and horses seem to be but slightly sensitive to the action of this poison.

A BASE, $C_{14}N_{20}O_4$, was isolated by GUARESCHI in 1887 from putrid fibrin. It occurs in the chloroform or ether extracts along with the base $C_{10}H_{13}N$, and is probably an amido-acid (see page 317).

A BASE, $C_7H_{18}N_2O_6$, was isolated by POUCHET in 1884. It is said to form short, thick prisms which become brown when exposed to light.

The PLATINOCHLORID, $(C_7H_{18}N_2O_6 \cdot HCl)_2PtCl_4$, crystallizes in prismatic needles which are insoluble in strong alcohol. For further details in regard to this base, see page 397.

A BASE, $C_{16}H_{23}N_2O_4$, was obtained by LEPIERRE (1894) in small quantity from poisonous cheese by precipitating, in the cold, with acetate of copper. It is crystalline, bitter, inodorous, and shows slight acid reaction to phenol-phthalein; is but slightly soluble in water, soluble in alcohol. It is dextro-rotatory, is precipitated by phosphomolybdic and picric acids, iodine in potassium iodide; not by tannin. When fed to guinea-pigs it produces diarrhoea. 0.05 g. injected intravenously into a rabbit had no effect. The hydrochloride is very soluble and forms large needles. The platinum and gold salts are crystalline.

TYROTOXICON has been obtained in poisonous cheese (VAUGHAN, WALLACE, WOLFF), in poisonous ice-cream (VAUGHAN, NOVY, SCHEARER, LADD), in poisonous milk (VAUGHAN, NOVY, NEWTON, WALLACE, FIRTH, SCHEARER), and in cream-puffs (STANTON). The methods of separating this poison and its effect upon animals have already been given with sufficient detail. Chemically, it is very instable. When warmed with water to about 90° , it decomposes. Hydrogen sulphid also decomposes it, therefore all attempts to isolate it by precipitation with some base, such as mercury or lead, and then removing the base with hydrogen sulphid, have failed. Its unstable character is illustrated by the fact that it may disappear altogether within twenty-four hours from milk rich in the poison which is allowed to stand in an open beaker.

With potassium hydrate it forms a compound which agrees in crystalline form, chemical reactions, and the per cent. of potassium which it contains, with the compound of diazobenzole and potassium hydrate. This substance is best obtained from milk containing tyrotoxinon as follows: The filtered milk, which is acid in reaction, is neutralized with sodium carbonate, agitated with an equal volume of ether, allowed to stand in a stoppered glass cylinder for twenty-four hours, the ether removed, and allowed to evaporate spontaneously from an open dish. The aqueous residue is acidified with nitric acid, then treated with an equal volume of a saturated solution of potassium hydrate, and the whole concentrated on a water-bath (this compound is not decomposed below 130°). On being heated the mixture becomes yellowish-brown, and emits a peculiar aromatic odor. On cooling the tyrotoxinon compound forms in beautiful, six-sided plates along with the prisms of potassium nitrate.

With equal parts of sulphuric and carbolic acids, pure tyrotoxinon gives a green coloration, but in whey the color varies from yellow to orange-red. This color-reaction may be used as a preliminary test in examining milk for tyrotoxinon. It is best carried out as follows: Place on a clean porcelain surface two or three drops each of pure carbolic and sulphuric

acids. Then add a few drops of the aqueous solution of the residue left after the spontaneous evaporation of the ether. If tyrotoxicon be present, a yellow to orange-red coloration will be produced. This test is to be regarded only as a preliminary one, for the coloration may be due to the presence of a nitrate or nitrite, or, as HUSTON and WEBER have shown, to butyric acid. The tyrotoxicon must be converted into the potassium compound and purified before the absence of nitrate or nitrite can be positively demonstrated. Moreover, the physiological test should always be made in testing for this poison.

With platinum chlorid in alcoholic solution tyrotoxicon forms a compound which explodes with great violence at the temperature of the water-bath. This also corresponds with the compound of platinum chlorid and diazobenzole.

Pure tyrotoxicon is insoluble in ether, and its extraction from alkaline solutions by this solvent is due to the presence of foreign matter, with which the poison is taken up by the ether.

The physiological action of this ptomaïn has been sufficiently discussed in a preceding chapter.

MYDALEIN (*μυδαλῆος*, putrid) is a poisonous base obtained in 1885 from putrefying cadaveric organs, liver, spleen, etc. (BRIEGER, II., 31, 48). Though it is apparently present on about the seventh day, it is unobtainable until about the third or fourth week. The method for its separation from the accompanying bases is given under saprin (page 342). It is liable to occur in the mercuric-chlorid filtrate, as well as in the precipitate, inasmuch as the double salt is insoluble only in perfectly absolute alcohol. In order to purify the platino-chlorid obtained as on page 342, it is repeatedly recrystallized from a very small quantity of lukewarm water. This base has not been obtained in sufficient quantity to permit of a complete determination of its composition. It is probably a diamine, containing four or five carbon atoms, and hence is nearly related to some of the diamines already described.

The PLATINOCHLORID, on analysis, gave: Pt = 38.74, C = 10.83, H = 3.23. It crystallizes in small needles, and is extremely soluble in water.

The HYDROCHLORID crystallizes with extreme difficulty, even on standing for some time in a desiccator. On exposure to the air it rapidly deliquesces.

Physiological Action.—Mydalein has an entirely specific action. Small quantities injected into guinea-pigs or rabbits produce, after a short time, a moistening of the under lip, and an abundant flow of secretion from the nose and eyes. The pupils dilate gradually to maximum, and become reactionless; the ear-vessels become strongly injected, and the body temperature rises 1° to 2° . The hairs bristle, and the animal occasionally shudders. Gradually the salivation ceases, the respiration and heart-action, which were at first hastened, now decrease, the temperature falls, the ears become pale, and the animal finally recovers. During the action of the poison the animal shows a tendency to sleep, and the peristaltic action of the intestines is heightened. Larger doses (0.050 gram) induce an exceedingly violent action, which invariably results in the death of the animal. On post-mortem the heart is found to be stopped in diastole, and the intestines and bladder contracted; otherwise nothing abnormal is observed.

A TOXIC BASE.—From human livers and spleens which were decomposing for two weeks in thorough contact with air there was isolated, besides cadaverin and putrescin, a small quantity of poisonous base (BRIEGER, II., 29, 48). The mercuric-chlorid precipitate was decomposed, and the hydrochlorids were precipitated by gold chlorid (to remove cadaverin, which is soluble), and the aurochlorid was then changed into platinum salt, whereby the insoluble putrescin platinochlorid was removed. In the mother-liquors from the putrescin salt an easily soluble platinum compound was separated, and found to contain 41.30 per cent. Pt. It crystallized in fine needles. The hydrochlorid formed small, readily deliquescent needles, and did not produce a precipitate in alcoholic platinum chlo-

rid. Injected into guinea-pigs and rabbits it induced an exalted peristaltic action of the intestines, which lasted several days, and produced in the animals, on account of the continuous evacuations, a condition of great weakness. No disturbance in the functions of the other organs was observed.

A BASE was isolated from decomposing haddock which were exposed for five days during summer in an open iron vessel. BRIEGER (I., 42) found in the aqueous mercuric-chlorid precipitate (see page 391) a base the hydrochlorid of which crystallized in well-formed, small needles. The platinochlorid likewise crystallized in beautiful needles, and gave, on analysis, 36.03 per cent. of Pt; 7.81 per cent. of N.

A substance of muscarin-like action was obtained by BRIEGER (I., 59) from putrefying gelatin, ten days at 35°, though in insufficient quantity to permit a determination of its character. The residue containing this substance gave, on distillation with alkali, only ammonia.

A BASE was obtained by BOCKLISCH (III., 52, 53) from herring which had undergone putrefaction for twelve days. It was found in the distillate, together with trimethylamin and dimethylamin, obtained by distilling the mercuric-chlorid filtrate, after the removal of the mercury, with sodium hydrate. The platinochlorid was easily soluble, and crystallized in large thin plates. On analysis it gave: Pt = 28.57, C = 22.34, H = 4.66. The hydrochlorid is easily soluble in water and in absolute alcohol, and besides with platinum gives only with phosphomolybdic acid a yellow precipitate which is soluble in excess, and with ammonia gives an immediate blue-color. It immediately reduces a mixture of ferric chlorid and potassium ferricyanid with formation of Berlin-blue; and similarly throws down metallic gold from solutions of gold chlorid.

From poisonous mussel, BRIEGER (III., 79) obtained an aurochlorid of a base crystallizing in needles. The quantity

isolated was insufficient for analysis. It is interesting because of its property of inducing salivation, a symptom which has been observed by SCHMIDTMANN and by CRUMPE in some cases of mussel-poisoning.

A BASE was obtained by GUARESCIHI and MOSSO (*Journ. für praktische Chem.*, **28**, 508) from fresh beef, in the alkaline ether-extract obtained by Dragendorff's method. It formed a yellowish alkaline fluid, of unpleasant odor, and after a time gave a deposit of microscopic crystals. The hydrochlorid gave the following reactions: Gold chlorid, yellow crystalline precipitate; platinum chlorid, precipitate; potassium iodid and iodine in hydriodic acid, kermes-red precipitate; phosphotungstic acid, nothing; phosphomolybdic acid, an abundant yellow precipitate; tannic acid, heavy, grayish precipitate, same with Mayer's reagent; picric acid, yellow precipitate; Marmé's reagent, precipitate soluble in excess; potassium bichromate, nothing; potassium permanganate and sulphuric acid, violet color; potassium ferrieyanid and ferric chlorid, Prussian-blue precipitate.

By giving a precipitate with tannin, and not with phosphotungstic acid, it resembles neurin.

CH. GRAM has studied the decomposition of yeast under the influence of an infusion of hay. The yeast was allowed to putrefy for fourteen days, and was then treated with zinc sulphate. The latter was precipitated by barium hydrate, and the filtrate after the removal of the barium by sulphuric acid was evaporated to dryness and extracted with absolute alcohol. The alcoholic solution was evaporated, and the residue again extracted with alcohol. The extraction-residue was taken up with water, and again subjected to the above treatment with zinc sulphate, barium hydrate, etc.

The filtrate was poisonous, and produced, in frogs, paralysis and stoppage of the heart in diastole. Addition of platinum chlorid and alcohol precipitated two bases. One of these, although possessing a curara-like action, did not affect the

heart. When its solution was heated for twenty-four hours on a water-bath it caused general paralysis and stoppage of the heart. The platinochlorid contained 38.05 per cent. of platinum.

The other base also possessed a slight curara-like action, and its platinochlorid gave, on analysis, 40.92 and 39.4 per cent. of platinum.

BRIEGER found a basic substance in small quantities in cultures of the staphylococcus pyogenes aureus on bouillon and beef-broth (II., 74). The hydrochlorid formed groups of colorless, non deliquescent needles. With platinum chlorid it yielded a double salt, crystallizing in needles, and containing 32.93 per cent. of Pt. For its reactions, see Table I.

From aqueous as well as alcoholic solutions of cultures of staphylococcus aureus, LEBER (1888) isolated a crystalline substance which he named *phlogosin*. The composition of this substance is not known. It does not seem to contain nitrogen, and inasmuch as it blackens silver it probably contains sulphur. It crystallizes in fine needles which are soluble in ether and in alcohol; difficultly soluble in water. It sublimes in needles. Alkalis precipitate it as amorphous yellow floccules which are soluble in acid and then can be recrystallized. With potassium ferricyanid and ferric chlorid it yields a blue color, and with potassio-mercuric, cadmic, and bismuth iodids precipitates which are soluble in excess. No precipitate is produced by gold or platinum chlorid, phosphotungstic or molybdic, tannic, or picric acid.

A small quantity applied to the conjunctiva produces intense inflammation, suppuration, and necrosis. Introduced into the anterior chamber it induces intense suppuration and keratitis. The substance is entirely distinct from the base obtained by BRIEGER, described above.

A. BASE—boiling-point about 284° —was obtained by BRIEGER (II., 61) from human livers and spleens which were putrefying for two to three weeks. It occurs in the mercuric-

chlorid filtrate, as described under saprin, page 342, together with some mydalein, trimethylamin, and hydrocarbon. The filtrate, after the mercury is removed by hydrogen sulphid, is evaporated to dryness, and finally the last traces of water are removed in a vacuum. The residue is then treated with absolute alcohol, and from this alcoholic solution the mydalein is precipitated by the addition of alcoholic mercuric chlorid. The trimethylamin is separated by distillation of the alkaline filtrate, previously deprived of its mercury by hydrogen sulphide; while the mother-liquor yields an oily mixture of hydrocarbons and bases. The latter were separated by fractional distillation, whereby only one of the bases was obtained in sufficient quantity for study. It boiled at about 284° , and gave with hydrochloric acid, on evaporation, a salt crystallizing in beautiful, long needles, which were very easily soluble in perfectly absolute alcohol. With gold chlorid and pieric acid it gave only oily products; with ferric chlorid and potassium ferrieyanid, an intense blue; with platinum chlorid, an extremely easily soluble double salt, which appeared under the microscope in the form of very fine needles; while from alcohol-ether the double salt slowly separated in thin plates which contained 30.36 per cent. of platinum. The free base showed a slight fluorescence. It is not poisonous, and, according to BRIEGER, is probably a pyridin-derivative.

Other non-poisonous bases were present in very small quantity in the mother-liquor described above, after the separation of the oily mixture.

PEPTOTOXIN.—By this name BRIEGER (I., 14–19) has designated a poisonous base which he has found in some peptons, and hence in the digestion of fibrin; in putrefying albuminous substances, such as fibrin, casein, brain, liver, and muscles. It is a well-known fact that animal tissues, in the early stages of putrefaction, possess strong toxic properties, even before the decomposition could have advanced far enough to effect a splitting up of the proteid and carbohydrate molecules. BRIEGER and others have tried to seek an explanation

of this toxicity by connecting it with an early peptonization of the proteids brought about by the action of ferments which are distributed throughout the tissues, and which begin their activity immediately after death. This poison has not been definitely isolated, but its general properties and action have been studied by BRIEGER, who prepared it by digesting fibrin for twenty-four hours with gastric juice at the temperature of the blood. The perfectly fresh pepton thus obtained was evaporated to a syrupy residue, and this was then extracted with boiling alcohol. The residue left on evaporation of the alcoholic solution was digested for some time with amyl alcohol, which on subsequent evaporation gave amorphous brownish masses. This extract was then purified by neutral lead acetate. The filtrate, after the removal of the lead by hydrogen sulphid, was repeatedly extracted with ether, then evaporated to dryness, and extracted as before, with amyl alcohol. This final extract was evaporated to drive off the alcohol, taken up with water, and filtered. The colorless aqueous solution thus obtained contains the poisonous substance, which, however, can only with extreme difficulty be brought to crystallization *in vacuo*.

SALKOWSKI in eight digestion-experiments with fresh fibrin obtained a poisonous extract in but one case. On the other hand, putrid fibrin or prolonged digestion, both implying bacterial activity, yields a poisonous product. Peptic digestion of serum-albumin, egg-albumin, and meat likewise gave negative results. In view of these facts as well as its presence in putrefying proteids, SALKOWSKI concludes that a peptotoxin (in the sense of BRIEGER) does not exist. That a poisonous product exists in fresh meat and in proteids on putrefaction seems to be well established. In 1891 BRIEGER ascribed a proteid nature to peptotoxin, excluding it from the bases. STADTHAGEN examined normal urine for peptotoxin, but failed to find it.

From animals with extensive burns KIJANITZIN has isolated, by means of Brieger's method for peptotoxin, from the urine, blood, and especially the organs, a substance resembling peptotoxin in chemical and physiological behavior. Atropin

antagonizes its action. The clinical symptoms in cases of extensive burns are closely allied to those observed in animals when a part or whole of the body is varnished. Rabbits die when $\frac{1}{8}$ — $\frac{1}{6}$ of the body-surface is varnished. Death in these cases has been explained by excessive loss of heat or by lack of excretion of waste-products of the skin. KIJANITZIN holds that the varnishing alters the chemical products of cells of the skin so that poisons are formed and carried throughout the body as in skin-burns.

Peptotoxin, when in its purest condition, as shown by its failure to give the biuret-reaction, possesses a neutral reaction. Its behavior to Millon's reagent is quite characteristic; it gives a white precipitate, which on boiling becomes intensely red. From this reaction, BRIEGER is inclined to regard this substance as a hydroxyl or an amido-containing-derivative of benzole. The ptomain can be extracted from acid as well as alkaline solution by amyl alcohol—more difficult in the cold than on heating. It is absolutely insoluble in ether, benzol, and chloroform; very soluble in water. It is not destroyed by boiling, by passing hydrogen sulphide, or by strong alkalis; but is destroyed, however, when the putrefaction lasts longer than eight days. For its behavior to reagents, see Table I.

Various observers have shown that pepton possesses a toxic action, and some have been led to regard this toxicity as not due to the pepton itself, but rather to the presence of this or some other ptomain. At least BRIEGER found one specimen of dry Witte's pepton to be perfectly harmless; whereas, the fresh pepton formed by fibrin-digestion possessed strong toxic powers. Moreover, this non-poisonous pepton when exposed to the action of gastric juice was found to yield the poisonous substance. The poisonous nature of proteids and the physiological action of this base will be described later.

PYOCYANIN, $C_{11}H_{11}N_2O$, is the coloring-matter of blue pus, and is produced by the action of the bacillus pyocyaneus. It was isolated by LEDDERHOSE (1887) and is said to be an anthracene-derivative. On contact with the air it is oxidized to pyoxanthose, a yellow substance. According to KUNZ, it con-

tains nitrogen and sulphur. The picrate is of a dark reddish-brown color; the platinum salt is black, and sometimes is obtained as glittering fine golden needles.

TABLE OF PTOMAINS.

Formula.	Name.	Discoverer.	Physiological action. ¹
C H ₅ N	Methylamin.	Bocklisch.	Non-poisonous.
C ₂ H ₇ N	Dimethylamin	Brieger.	" "
C ₃ H ₉ N	Trimethylamin.	Dessaigues.	" "
C ₂ H ₅ N	Spermin (?).	Kunz.	" "
C ₂ H ₇ N	Ethylamin.	Hesse.	" "
C ₄ H ₁₁ N	Diethylamin.	Bocklisch.	" "
C ₆ H ₁₅ N	Triethylamin.	Brieger.	" "
C ₃ H ₉ N	Propylamin.	"	"
C ₄ H ₁₁ N	Butylamin.	Gautier & Mourgues.	Poisonous(?).
C ₅ H ₁₁ N (1)	Tetanotoxin.	Brieger.	Poisonous.
C ₆ H ₁₃ N	Amylamin.	Hesse.	"
C ₆ H ₁₅ N	Hexylamin.	"	"
C ₇ H ₁₇ N	10-hydrofufidin.	Gautier & Mourgues.	"
C ₈ H ₁₇ N	Collidin (?).	Neneki.	"
C ₈ H ₁₁ N	Pyridin-base (?).	O. de Confnek.	"
C ₈ H ₁₃ N	Hydrocollidin (?).	Gautier and Etard.	Poisonous.
C ₉ H ₁₃ N	Parvolin (?).	" " "	"
C ₁₀ H ₁₅ N	Unnamed.	Guareschi & Mosso.	Poisonous.
C ₁₀ H ₁₅ N	Pyridin-base (?).	O. de Confnek.	"
C ₁₂ H ₂₁ N	Unnamed.	Deléznier.	"
C ₂ H ₈ N ₂	Ethylidenediamin (?)	Brieger.	Poisonous.
C ₃ H ₈ N ₂	Anthracin.	Hoffa (1889).	"
C ₃ H ₈ N ₂	Trimethylenediamin (?).	Brieger.	Poisonous.
C ₄ H ₁₂ N ₂	Putrescin.	"	Not very poisonous.
C ₅ H ₁₄ N ₂	Cadaverin.	"	" " "
C ₅ H ₁₄ N ₂	Neuridin.	"	Non-poisonous.
C ₅ H ₁₄ N ₂	Saprin.	"	" "
C ₆ H ₁₆ N ₂	Hexamethylenediamin.	Garcia.	"
C ₇ H ₁₆ N ₂	Unnamed.	Morin.	Non-poisonous.
C ₁₀ H ₂₅ N ₂ (?)	Susotoxin.	Novy.	Poisonous.
C ₂ H ₇ N ₃	Methyl-guanidin.	Brieger.	"
C ₁₀ H ₂₇ N ₃	Morrhuin.	Gautier & Mourgues.	Diuretic, etc.
C ₁₃ H ₂₉ N ₄	Unnamed.	Oser.	"
C ₁₇ H ₃₈ N ₄	"	Gautier & Etard.	"
C ₂₅ H ₅₂ N ₄	Asellin.	Gautier & Mourgues.	Poisonous.
C ₅ H ₁₃ N O	Neurin.	Brieger.	"
C ₈ H ₁₁ N O	Mydin.	"	Non-poisonous.
C ₆ H ₁₁ N O ₂	δ-amido-valerianic acid.	E. & H. Salkowski.	" "
C ₆ H ₁₅ N O ₂	Chollin.	Brieger.	Poisonous.
C ₈ H ₁₈ N O ₂	Mydatoxin.	"	"

¹ Only those bases are here denoted as poisonous which possess a decided toxicity.

TABLE OF PTOMAINS—*Continued.*

Formula.	Name.	Discoverer.	Physiological action. ¹
$C_6 H_{13} N O_2$	Unnamed.	Brieger, 1888 (tetanus cult.).	Non-poisonous.
$C_6 H_{15} N O_2$	Mytilotoxin.	Brieger.	Poisonous.
$C_7 H_{17} N O_2$	Gadinin.	"	"
$C_7 H_{17} N O_2$	Typhotoxin.	"	"
$C_7 H_{17} N O_2$	Unnamed.	"	"
$C_{14} H_{14} N_2 O$	Pyocyanin.	Ledderhose.	Non-poisonous.
$C_6 H_{13} N O_3$	Betain.	Brieger.	" "
$C_5 H_{15} N O_3$	Muscarin.	"	Poisonous.
$C_9 H_{13} N O_3$	Morrhuae acid.	Gautier & Mourgues.	
$C_6 H_{12} N_2 O_4$	Unnamed.	Pouchet.	Poisonous.
$C_{13} H_{30} N_2 O_4$	Tetaniin.	Brieger.	"
$C_{14} H_{20} N_2 O_4$	Unnamed.	Guareschi.	
$C_{16} H_{22} N_2 O_4$	"	Lepierre.	Poisonous.
$C_7 H_{18} N_2 O_5$	"	Pouchet.	"
	"	"	"
	Tyrototoxin.	Vaughan.	"
	Mydalein.	Brieger.	"
	Spasmotoxin.	"	"
	Λ diamin (?).	" (tetanus cult.)	"
	Peptotoxin.	"	"
	Phlogosin.	Leber.	Inflammatory.

¹ Only those bases are here denoted as poisonous which possess a decided toxicity.

For GRIFFITH'S bases, see page 320.

CHAPTER XIII.

CHEMISTRY OF THE LEUCOMAINS.

UNDER this head are classed those basic substances which are found in the living tissues, either as the products of fermentative changes, other than those of bacteria, or of retrograde metamorphosis. Most of these substances have already been known for many years, though their real significance as alkaloidal bodies and their relation to the functional activities of the animal organism have been but little understood, or rather they have not been brought together under the leading conception that they are alkaloidal products of physiological change. The first attempt at the systematic study and generalization of these basic substances was made by GAUTIER, who applied to them the name leucomains, a term derived from the Greek, λευκωμα, signifying white of eggs. Under this name he includes all those basic substances which are formed in animal tissues during normal life, in contradistinction to the ptomaines or basic products of putrefaction. The distinction between vegetable and animal alkaloids is not very well defined, and, in fact, there seem to be reasons for considering their formation as due to the same causes which bear an intimate relation to the physiology of the cells and tissues of both kingdoms. Thus, vegetable tissues are known to contain not only what are ordinarily designated as ptomaines, such as cholin, but also leucomains, as hypoxanthin, xanthin, etc. Indeed, in this latter group must be placed, on account of their relation to xanthin, those well-defined alkaloidal bases, caffeine and theobromin. Not only are the representatives of these two divisions of basic substances common to both kingdoms, but their parent bodies, lecithin, nuclein, etc., are known to occur in both, thus giving rise to the same bases on decomposition.

So far as the genesis of most of the leucomains is con-

cerned, we know very little, though GAUTIER is of the belief that they are being formed continuously and incessantly in the animal tissues side by side with the formation of urea and carbonic acid, and at the expense of the nitrogenous elements. It is quite probable, as KOSSEL has pointed out, that some of these products are in themselves antecedents of end-products of metabolism. This is unquestionably true of the imido-group, which exists in the adenin and guanin molecules, and through vital or putrefactive processes is split off, giving rise to ammonia, which in turn serves to form urea. BOUCHARD has sought an explanation of the presence of these bases in the urine, by supposing that they were originally formed in the intestinal tract, from which they were absorbed into the system, to be subsequently eliminated by the kidneys. This view has also been brought forward by SCHÄR (1886) who holds that these bases, which may be formed by putrefactive changes in the intestinal tract, are absorbed into the circulatory system, whence they may be partly eliminated by the kidneys, or may be partly deposited in the tissues themselves.

The views of BOUCHARD and SCHÄR have, to a certain extent, been confirmed by the investigations of UDRÁNSZKY and BAUMANN, who showed that the well-known ptomaines cadaverin and putrescin occur in the urine in cystinuria, and are formed by putrefactive changes induced in the intestinal tract, probably by specific micro-organisms. Under this same head fall the recent observations of WOLKOW and BAUMANN, that alkapton is produced from tyrosin by similar changes in the intestines. The production of intestinal products, their absorption and excretion by the kidneys, is likewise seen in such well-known compounds as phenol, indol, skatol, etc. The origin of the true leucomaines cannot, however, be accounted for in this manner, for they are indissolubly connected with the metabolism of the cell itself, and are, therefore, formed in the tissues and organs proper, especially those rich in nucleated cells.

Another source of the nitrogenous bases must not be lost sight of, and that is protoplasm itself. The researches of DRECHSEL, SIEGFRIED, and SCHULZE have shown that

nitrogenous bases do result from the decomposition of animal and vegetable proteids (see p. 367).

The leucomains proper can be divided into two distinct and well-defined groups: (1) the Uric acid Group, and (2) the Creatinin Group.

The first of these divisions contains a number of well-known bases which are closely related to uric acid. The order in which they will be described is as follows:

Adenin,	$C_5H_5N_5$.
Hypoxanthin,	$C_5H_4N_4O$.
Gnanin,	$C_5H_5N_5O$.
Xanthin,	$C_5H_4N_4O_2$.
(Uric Acid,	$C_5H_4N_4O_3$.)
Heteroxanthin,	$C_6H_6N_4O_2$.
Methylxanthin,	$C_6H_6N_4O_2$.
Paraxanthin,	$C_7H_8N_4O_2$.
Carnin,	$C_7H_8N_4O_3$.
Episarkin,	$C_4H_6N_3O(?)$.
Pseudoxanthin,	$C_4H_5N_5O$.
Cytosin,	$C_{21}H_{30}N_{16}O_4$.
Gerontin,	$C_5H_{14}N_2$.
Spermin,	$C_2H_5N(?)$.

The members of the second group have all been discovered by GAUTIER, and by him are regarded as allied to creatin and creatinin. These two substances, especially the latter, have been hitherto regarded as strongly basic in character, but SALKOWSKI has recently shown that creatinin, when perfectly pure, possesses little or no alkaline reaction, and, moreover, does not combine with acids. The bases in this group are:

(Creatinin,	$C_4H_7N_3O$.)
(Creatin,	$C_4H_9N_3O_2$.)
Criso-creatinin,	$C_5H_8N_4O$.
Xantho creatinin,	$C_5H_{10}N_4O$.
Amphi-creatin,	$C_9H_{19}N_7O_1$.
Base,	$C_{11}H_{24}N_{10}O_5$.
Base,	$C_{12}H_{25}N_{11}O_5$.

Besides these two general classes of leucomaïns, there may be made a third class of undetermined leucomaïns, embracing those bases which have been observed, but studied more or less incompletely, in the various physiological secretions of the body.

LEUCOMAÏNS OF THE URIC-ACID GROUP.

ADENIN, $C_5H_5N_5$, which was discovered by KOSSEL in 1885, forms the simplest member of the uric-acid group of leucomaïns, and as such it deserves special attention, inasmuch as it shows most clearly the relation that exists between hydrocyanic acid and the members of this group. This base is apparently formed by the polymerization of hydrocyanic acid—a view that is confirmed, at least in part, by the fact that on heating with potassium hydrate to 200° it yields a large quantity of potassium cyanide. Moreover, by the action of reducing agents it is converted into a substance similar to, if not identical with, azulmic acid. It has not been prepared synthetically, though GAUTIER has claimed to have synthesized two closely related bodies, xanthin and methyl-xanthin, by simple heating of hydrocyanic acid in a sealed tube in contact with water and a little acetic acid. The molecular weight of adenin has been determined by KOSSEL according to Beckmann's method. The formula of adenin is, therefore, not a multiple of that given above.

This base was first prepared from pancreatic glands—hence the term adenin, which is derived from the Greek word *αδην*, meaning a gland. It has since been shown to occur together with guanin, hypoxanthin, etc., as a decomposition-product of nuclein, and, therefore, it may be obtained from all tissues and organs, animal or vegetable, rich in nucleated cells. Accordingly, it has been found in the kidneys, spleen, pancreatic, thymus, and lymphatic glands, in beer-yeast, in spermatie fluids, but not in testicles of the steer; occurs also in tea-leaves. In the latter adenin appears to exist in a preformed condition, since it can be extracted without the use of acid reagents. (Hypoxanthin absent, KRÜGER, 1896.) Tea-

extract yields about 6 grams of adenin per liter (KRÜGER). The thymus gland, as a prototype of embryonic, highly cellular tissue, yields a considerable amount of adenin, but no xanthin (INOKO); that from a calf, for instance, was found by SCHINDLER to contain 0.18 per cent. The thymus nucleinic acid yields only adenin, no xanthin (KOSSEL). There are, therefore, no tissues known in which xanthin and guanin are exclusively present—and the sarkin-bases absent (KOSSEL). It has also been observed in the liver and urine of leucoeythæmic patients (STADTHAGEN); its occurrence in this disease will be readily understood when it is remembered that leucoeythæmia is characterized by the presence in the blood of an unusual proportion of the nucleated white blood-corpuscles, which, owing to various unfavorable conditions, become destroyed in time, and the contained nuelein, as a result, splits up into adenin and guanin. These two bases may, therefore, be expected in all pathological conditions where there is an abnormal accumulation of pus. Indeed, as early as 1865, NAUNYN extracted from pus, obtained from the pleural cavity, a considerable quantity of a substance which was probably either adenin or guanin, or both. Neither uric-acid nor xanthin bases are present in fresh human blood (100–300 c.c.); both are present in exudates and transudates (JAKSCH). Adenin does not occur, or only in minute traces, in meat-extract; and in this it resembles guanin, which is present only in traces. This may be due to the fact that adenin and guanin are readily converted into hypoxanthin and xanthin respectively, as has been shown in the putrefaction-experiments of SCHINDLER. This conversion of adenin and guanin into hypoxanthin and xanthin takes place in the pancreas immediately after death, so that the amount of adenin found may be quite small. They may be considered as transitional products of cell-metabolism, the amido-group contained in each readily being replaced by oxygen, and giving rise to ammonia, and this in turn to urea. KOSSEL, however, explains this fact on the ground that the muscle-tissue is very poor in nucleated cells, *i. e.*, in nuelein. It would seem that the muscle-cell in losing the morphological character of a cell

has also suffered a corresponding loss in its chemical properties. For while the decomposition products of nuclein—hypoxanthin, xanthin, phosphoric acid, etc.—are found in the muscle-tissue, they do not exist in combination as they do in the nuclein-molecules. This is seen in the fact that the bases exist in the free condition, since they can be extracted by water; and again, the phosphoric acid is present in the muscle-tissue, not in organic combination, but as a salt. In the nucleated cell, adenin, guanin, etc., do not exist in the free condition, but form, in part at least, with albumin and phosphoric acid, a loose combination which is readily decomposed by the action of acids at the boiling temperature. This same change takes place spontaneously after death.

There can be no doubt that adenin and guanin play an important part in the physiological function of the cell-nucleus, which, from recent observations, appears to be necessary to the formation and building up of organic matter. It is now known that non-nucleated cells, though capable of living, are not capable of reproduction. We must look, therefore, to the nucleus as the seat of the functional activity of the cell—indeed, of the entire organism. Nuclein, the parent-substance of adenin and guanin, is the best known and probably most important constituent of the nucleus, and as such it has been already credited with a direct relation to the reproductive powers of the cell. This chemical view has recently been confirmed by ZACHARIAS, who showed that chromatin of histologists is identical with nuclein. LIEBERMANN has questioned nuclein as being the source of xanthin-compounds, but in this he is not supported by the mass of evidence.

LILJENFELD, in his study of the chemistry of leucocytes, has shown that the nuclei of these cells contain a complex body, nucleohiston, which is decomposed by acids into histon and leuconuclein. The latter in turn can be decomposed into albumin and nucleinic acid, which on heating with mineral acids yields phosphoric acid, and the nuclein-bases (adenin, hypoxanthin) and unknown products. As KOSSER has pointed out, it is probable that ordinary nucleinic acid is

a mixture of several, since two or more nuclein-bases form on decomposition. The nucleinic acid from the thymus gland, adenylic acid, yields on decomposition only one nuclein-base—adenin. Another base, however, can be obtained. See Cytosin.

The method employed by KOSSEL for the preparation of adenin is as follows: The finely divided pancreatic glands are heated to boiling for three or four hours with a large quantity of dilute sulphuric acid (0.5 per cent. by volume of concentrated acid), and the acid solution thus obtained is treated with a slight excess of hot concentrated baryta-water. The excess of baryta is removed by carbonic acid, and the solution is then filtered; the filtrate is concentrated to a small bulk, about 100 c.c., rendered alkaline with ammonium hydrate, and finally precipitated with an ammoniacal solution of silver nitrate. The precipitate, consisting of the silver compound of the xanthin-bodies, is partially dried by spreading over porous porcelain plates; then dissolved in warm nitric acid of specific gravity 1.1, to which a little urea has been added to prevent the formation of hypoxanthin should traces of nitrous acid be present. (KRÜGER recently, 1896, showed that even large excess of urea does not prevent this change of adenin into hypoxanthin.) The filtered acid solution, treated with silver nitrate, on cooling gives a deposit of silver salts of hypoxanthin, guanin, and adenin, which is filtered off and thoroughly washed. The adenin separates out almost quantitatively if a little silver nitrate solution is added. The filtrate contains any xanthin silver-compound that may be present. The washed precipitate of the silver salts is suspended in water, nitric acid added, decomposed with hydrogen sulphid (ammonium sulphid, or, better, hydrochloric acid, may be used), and the clear filtrate is concentrated on a water-bath to a small volume. It is then saturated with ammonium hydrate and digested on a water-bath for some time, whereby adenin and hypoxanthin go into solution, while the guanin remains undissolved (see p. 423). Some guanin, however, is dissolved, as has been shown by WILFF. From the ammoniacal solution on partial concentration and

subsequent cooling, the adenin crystallizes out first, whereas the more soluble hypoxanthin remains in solution. If the adenin is still colored, it can be purified by dissolving in water and boiling with animal charcoal. The hot aqueous solution is then rendered very slightly alkaline with ammonium hydrate and allowed to cool; adenin crystallizes out, and can be still further purified by recrystallization from water.

In the examination of meat-extracts, according to Neubauer, the solution is precipitated with basic lead acetate, avoiding an excess, filtered, and from the filtrate the lead is removed with hydrogen sulphid. The filtrate is concentrated on a water-bath to a thin syrup, and allowed to stand for two or three days to allow creatin to crystallize. The fluid is decanted, the crystals washed with 90 per cent. alcohol, the combined liquids evaporated on a water-bath to expel alcohol, then rendered ammoniacal, and precipitated with silver nitrate as above (BALKE).

Ammonium sulphid has been employed by SCHINDLER, and sodium sulphid by BALKE, in place of hydrogen sulphid, in decomposing the silver compounds of the above bases. BRUHNS recommends instead warming with very dilute hydrochloric acid, especially if guanin is present. The solution can then be neutralized with NaHCO_3 , using methyl-orange as indicator, and the adenin separated from hypoxanthin by the pierie-acid method described below.

Another method for the separation of adenin from hypoxanthin is based upon the behavior of the nitrates of these bases in aqueous solution. From concentrated aqueous solutions of the nitrates free hypoxanthin crystallizes out first, because the nitrate is decomposed; whereas, adenin, which is a stronger base, remains in combination with the acid in solution.

A still more recent method for the separation of adenin and hypoxanthin is based on the fact that adenin is precipitated from the solution of the base or salt by copper sulphate and sodium hyposulphite (even 1:50,000), whereas hypoxanthin, in even 0.5 per cent. solution, is not precipi-

tated in the cold. On heating, however, the enprous compound forms (KRÜGER).

SCHINDLER determines adenin and hypoxanthin indirectly. The ammoniacal solution which is filtered from the insoluble guamin is evaporated to dryness on a weighed platinum dish, dried at 110° , and weighed. A nitrogen determination is now made of the mixed bases, and from these data the proportion of each is calculated.

The best source of adenin, however, is the mother-liquor from tea-leaves, which yields from 3 to 6 g. per litre. KOSSEL first isolated adenin from this source in 1886. The mother-liquor was precipitated with basic lead acetate; the lead removed from the filtrate by the addition of H_2SO_4 . The solution was then rendered ammoniacal and precipitated with ammoniacal silver solution. The precipitate was then treated as described above (p. 419). The method for its extraction from this source, as modified by KRÜGER, avoids drying the silver compounds on porous plates, and also the recrystallization from hot nitric acid. The mother-liquor is diluted with five volumes of water and the humin substances are then precipitated by the addition of sulphuric acid. The liquid is then separated by decantation and filtration, rendered strongly alkaline with ammonium hydrate, and the bases precipitated with ammoniacal silver solution. The voluminous precipitate, after standing twenty-four hours, is filtered through a plaited filter, washed with cold, then with hot water, and finally is allowed to remain on the filter for one to two days. It is then removed from the filter, decomposed with concentrated hydrochloric acid, filtered, and the filtrate neutralized with sodium hydrate. The solution is then dissolved with animal charcoal, and concentrated by evaporation. The crystalline mass which separates out on cooling is filtered off with the aid of a pump and washed with water. By repeating the process additional crystals are obtained from the mother-liquor. The rough product is purified by dissolving in one per cent. hydrochloric acid with the aid of heat, decoloring with charcoal, after which, on cooling, the almost pure adenin hydrochlorid separates out. On

further concentration and cooling additional crystals are obtained. To purify the crystals still further they are dissolved in hot water, and the hot solution is precipitated by addition of ammonium hydrate. After twenty-four hours the crystals are filtered off, dissolved, and again precipitated with ammonia.

For the application of the new copper method, which is easier and cheaper than the silver method for the extraction of adenin from tea extracts, see KRÜGER, *Zeitschr. f. physiol. Chem.*, **21**, 274, 1896. Adenin is best purified from the crude bases by crystallization as the sulphate.

By far the best method for the quantitative separation of adenin and hypoxanthin is the pierate method of BRUHNS. The solution of the salts of the bases, preferably as nitrates or sulphates, must be neutral or faintly acid; excess of alkali or acid interferes. Such a solution can be obtained by evaporating the filtrate from the guanin in KOSSEL's method (page 419), and dissolving the residue in nitric acid; this is neutralized with sodium carbonate, using methyl-orange as indicator. On the addition of excess of sodium pierate the adenin is thrown down as a clear yellow flocculent precipitate. If the precipitation is made at the boiling temperature, on cooling the adenin salt separates in a crystalline condition and is more easily filtered and washed. After standing fifteen minutes the precipitate is filtered off by the aid of a suction-pump on a weighed filter, washed with cold water, and dried at 100°. As a correction for the solubility of the adenin pierate, 2.4 mg. per 100 c.c. filtrate can be added to the calculated amount of adenin.

The hypoxanthin pierate is not very soluble, as was supposed by BRUHNS, so that if the amount present is large and the precipitate not filtered at once, somewhat higher results will be obtained (WULF). In the filtrate, however, it may be estimated according to the method described on page 447.

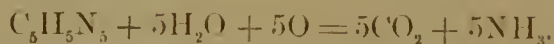
For the estimation of adenin or hypoxanthin by KRÜGER's copper method see page 448.

Adenin, when crystallized from warm or impure solutions, is obtained either as an amorphous substance, pearly plates,

or in the form of very small microscopic needles; from dilute cold solutions it separates in long, needle-shaped crystals containing three molecules of water. This water of crystallization is lost on exposure to the air or on heating to 53° , and the crystals become opaque. By precipitating a concentrated solution of the hydrochlorid with ammonia adenin may be obtained as anhydrous, small whetstone-shaped crystals, which, recrystallized from hot water, form large, regular, four-sided pyramids, single or bur-shaped. It is soluble in about 1086 parts of water at the ordinary temperature; more easily in hot water, from which on cooling it recrystallizes. The aqueous solution possesses a neutral reaction. The free base is insoluble in ether, chloroform, and alcohol; soluble in glacial acetic acid, and somewhat in hot alcohol. It dissolves readily in mineral acids, yielding well crystallizable salts. The fixed alkalis dissolve it with ease, but on neutralization of the solution it is reprecipitated; from such solutions in alkalis anhydrous large crystals are thrown down by acetic or carbonic acid (KRÜGER). In aqueous ammonium hydrate it is more readily soluble than guanine (which is insoluble, SCHINDLER; somewhat soluble, WULFF), and more difficultly soluble than hypoxanthin—a fact which is made use of to effect a separation from these bases. It is but slightly soluble in sodium carbonate.

Adenin can be heated to 278° without melting; at this temperature it becomes slightly yellow, and yields a white sublimate. It can be completely volatilized without decomposition, by heating on an oil bath to 220° ; the sublimate consists of pure, white, plumose needles of adenin, but at 250° partial decomposition occurs, and some hydrocyanic acid forms. When heated with potassium hydrate to 200° on an oil bath, it yields a considerable quantity of potassium cyanid. Adenin is quite indifferent to the action of acids, alkalis, and even oxidizing agents. Thus, it may be boiled for hours with baryta, potash, or hydrochloric acid, without suffering decomposition. But when heated with dilute hydrochloric acid at 135° for several days, or with concentrated hydrochloric acid, in a sealed tube at a temperature exceeding 100° ,

adenin is completely decomposed, with formation of carbonic acid and ammonia:



On heating adenin with concentrated hydrochloric acid to 180° – 200° for 12–14 hours, KRÜGER obtained ammonia, carbon dioxide, carbon monoxid, and glyccoll. The carbon monoxid results from the splitting up of formic acid. This decomposition is strictly analogous to that of hypoxanthin, xanthin, etc.



The free base, as well as benzoyl-adenin, is unaffected by the weak oxidizing action of potassium permanganate, but on stronger oxidation it is wholly destroyed. Bromine-water produces in aqueous solutions of adenin an oily precipitate, which, on contact with potassium hydrate or ammonia, gives a beautiful red or violet color. Sodium amalgam and zinc chlorid appear to have no action; but on boiling with zinc and hydrochloric acid it yields a very unstable reduction-product, which in the presence of oxygen, in alkaline solution, first assumes a red color, and finally throws down a reddish-brown precipitate. This brown substance appears to be identical with azulmic acid, which has been known for a long time as a product of the polymerization of hydrocyanic acid.

Adenin and hypoxanthin do not give the xanthin reaction; that is to say, when adenin is evaporated on a water-bath with dilute or fuming nitric acid it gives a white residue which fails to give any coloration with sodium, ammonium, or barium hydrate (xanthin-reaction). Similarly, it does not give the so-called Weidel's reaction on heating with fresh chlorine water and a trace of nitric acid as long as gas is given off, then evaporating to dryness on a water bath and exposure of the residue to an ammoniacal atmosphere. In this respect it resembles hypoxanthin, which, when pure, does not answer to either of these tests. When either of these bases, however, is evaporated on a water-bath with bromine-water and nitric acid a residue is obtained which with alkalis

is colored red (KOSSEL). Another test for adenin, which is given also by hypoxanthin, but not by guanin, caffein, and episarkin, is as follows: The substance to be tested is digested for half an hour with zinc and hydrochloric acid in a test-tube on a water bath. If adenin is present, the solution will assume on standing, more rapidly on shaking, a ruby-red coloration, which later on turns into a brownish-red. This reaction depends upon the formation of a reduction product, which, owing to its unstable nature, is soon oxidized by the oxygen of the atmosphere into a brownish, amorphous substance, apparently identical with azulmic acid.

Ferrie chlorid imparts to an aqueous solution of adenin an intense red color which is not affected by heating. Copper sulphate produces an amorphous grayish-blue precipitate, which is easily soluble in dilute acids and ammonia. The light-blue solution in fixed alkalis on warming gives a precipitate of copper oxide.

DRECHSEL'S reaction. In 1892 DRECHSEL showed that certain xanthin bases are precipitated by an ammoniacal solution of cuprous chlorid; or from fixed alkaline solution by Fehling's solution in the presence of a reducing-substance. In addition to uric acid, which has been known to give this reaction, xanthin, guanin, hypoxanthin, creatin, and creatinin, the latter in boiling, reacted. BALKE applied the test to fixed alkaline solutions, using Fehling's solution, and as reducing substances hydroxylamin hydrochlorid, or dextrose. He found that adenin, hypoxanthin, xanthin, heteroxanthin, paraxanthin, earnin, protamin, and uric acid gave precipitates, whereas theobromin and caffein did not. KRÜGER employed copper sulphate and sodium bisulphite, the advantage being that the precipitation can take place in neutral, acid, or alkaline solutions. The results differ somewhat with the kind of reducing agent employed. Thus, copper sulphate and sodium bisulphite precipitate uric acid, adenin, methyladenin, hypoxanthin, guanin, also dimethyl hypoxanthin from cold concentrated solution; theobromin, caffein, creatin, creatinin are not precipitated. With copper sulphate and sodium

hyposulphite adenin, methyl-adenin, and guanin are readily precipitated; hypoxanthin only on heating (separation from adenin), whereas the other six compounds are not precipitated. The precipitates are soluble in excess of sodium hyposulphite.

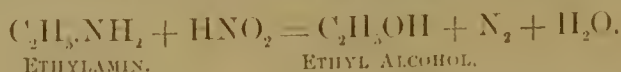
Adenin and hypoxanthin can, therefore, be completely precipitated, especially by the aid of heat, from their solution by copper sulphate and sodium bisulphite. Hence this reagent could be used as a substitute for ammoniacal silver solutions in the method of separation and even of estimation by determining either the amount of copper or of nitrogen by Kjeldahl's method. The adenin precipitate is colorless and gelatinous; changes on exposure to a light or brownish green, and on drying it becomes dark green. It is easily soluble in mineral acids, especially nitric; slowly soluble in hot acetic acid. It is not decomposed with sodium hydrate; readily decomposed with alkali sulphids, and is readily soluble in ammonia. It is soluble in about 200,000 parts of hot water.

On treatment with nitrous acid it is converted into hypoxanthin according to the equation :



KOSSEL obtained 72 per cent. of the theoretical yield. Since then KRÜGER, by modifying the experimental conditions, adding sodium nitrite in small portions to a solution of adenin in dilute sulphuric acid at 70°, obtained an almost quantitative conversion.

This formation of hypoxanthin from adenin is analogous to STRECKER's transformation of guanin into xanthin by a similar action of nitrous acid (see Guanin). In both cases the change of a highly nitrogenized into a less nitrogenized body is accomplished by replacing an NH group by O. The change is somewhat analogous to that seen in the conversion of primary amines into primary alcohols. Thus,



In the extraction of adenin from the mother-liquor of tea-leaves after removal of caffen, if urea is not added to the

nitric acid, nearly one-half of the adenin may be converted into hypoxanthin. By processes of putrefaction adenin is converted into hypoxanthin and guanin into xanthin (SCHINDLER). A similar conversion of adenin and guanin takes place rapidly in the pancreas after death. The change is, therefore, somewhat analogous to that produced by nitrons acid. Adenin undergoes this decomposition much more rapidly than the other xanthin compounds.

The ease with which adenin and guanin are oxidized outside of the organism shows that similar changes may take place within the cell-nucleus proper. For we know that every cell is endowed with an enormous reducing power, and hence it is not difficult to see how the oxygen-free adenin can be readily converted into a body or bodies which greedily take up oxygen. We must, therefore, look upon adenin and guanin not only as the antecedents of hypoxanthin and xanthin, but also as intermediate products which, when they form in the cell, may give rise to important chemical processes, especially those of a synthetic nature. It is highly probable that the study of the decomposition-products of nuclein will explain to us many of the metabolic changes in the organism, and throw additional light upon the migration of the amido-group from the proteid molecule to the amido-acids and urea-derivatives. Thus, the formation of xanthin from guanin represents the conversion of a guanidin residue into a urea residue. A similar change is probably effected in the transformation of adenin into hypoxanthin.

Adenin unites with bases, acids, and salts. The salts of adenin with mineral acids can be recrystallized, thus differing from the corresponding salts of guanin and hypoxanthin, which are dissociated by the action of water. The solutions of the salts, however, show an acid reaction to litmus, but not to methyl-orange.

The hydrochlorid, $C_5H_5N_5.HCl + \frac{1}{2}H_2O$, forms colorless, transparent, strongly refracting crystals. One part of the anhydrous salt is soluble in 41.9 parts of cold water. Microscopically it is distinct from that of hypoxanthin and adenin-

hypoxanthin. From the composition of the gold salt it is highly probable that a hydrochlorid, $C_5H_5N_5 \cdot 2HCl$, exists analogous to that of guanin.

The nitrate, $C_5H_5N_5 \cdot HNO_3 + \frac{1}{2}H_2O$, crystallizes from the aqueous solution in fine, stellate needles. One part of the dry salt dissolves in 110.6 parts of water.

The sulphate, $(C_5H_5N_5)_2 \cdot H_2SO_4 + 2H_2O$, can be obtained from the aqueous solution in two different crystalline forms. This may possibly be due to the presence of adenin hypoxanthin compound (BRUNN). It is easily soluble in hot water, and at the ordinary temperature it is soluble in 153 parts of water.

The oxalate, $C_5H_5N_5 \cdot C_2H_2O_4 + H_2O$, is obtained by dissolving adenin in hot, dilute, aqueous oxalic acid, from which solution, on cooling, it separates as a voluminous difficultly soluble precipitate of roundish masses which are composed of long, delicate needles. The oxalates of guanin, hypoxanthin, and xanthin are more easily soluble than that of adenin, and exhibit, moreover, a different appearance.

Adenin bichromate, $(C_5H_5N_5)_2 \cdot H_2Cr_2O_7$. This compound separates in a few hours from a mixture of adenin and chromic acid solutions in well-formed yellowish-red crystals (BRUNN). According to KRÜGER, it forms six-sided plates, is easily soluble in hot water, difficultly in cold, and is unchanged by heating to 150° . The corresponding salt of guanin readily dissociates.

Adenin metaphosphate, $C_5H_5N_5 \cdot HPO_3$. According to KOSSEL, adenin is not precipitated with metaphosphoric acid, but this is not strictly true. Aqueous, or even cold saturated solutions of adenin give on the addition of a few drops of metaphosphoric acid an amorphous precipitate, appearing under the microscope as fine round granules or extremely thin membranous masses. It has not been obtained in a crystalline condition. Like the corresponding guanin compound, it is difficultly soluble in cold water. It is easily soluble in alkalis and in ammonia; is more or less soluble in dilute acids according to the concentration, and is soluble in excess of

metaphosphoric acid. Hence a strongly acid, not too concentrated, solution of adenin is not precipitated (WULFF). Adenin is precipitated less completely than guamin, whereas hypoxanthin does not give a difficultly soluble metaphosphate.

The chloracetate, $C_5H_5N_5 \cdot ClCH_2 \cdot CO_2H$, was prepared by KRÜGER by adding an excess of chloracetic acid to a hot aqueous solution of adenin. On cooling it crystallizes in right-angled plates and in stellate four-sided prisms. It is easily soluble in water and in hot aqueous alcohol; difficultly in cold alcohol. At 162° – 163° it melts, giving off hydrochloric acid and forming a yellowish-red fluid which gradually becomes intensely red.

Potassium ferro- and ferricyanid produce no precipitate in a solution of adenin, but if acetic acid is then added the former gives rise to a precipitate of thin plates; the latter, a precipitate of light-brown crystals grouped in bunches (KRÜGER). According to BRUNN, adenin gives with potassium ferricyanid brownish-green needles.

The picrate, $C_5H_5N_5 \cdot C_6H_2(NO_2)_3OH + H_2O$, is thrown down as a bright-yellow flocculent precipitate when aqueous solutions of adenin salts are treated with sodium picrate. Recrystallized from hot water it forms bright-yellow, very voluminous bunches of long, fine needles, which, on drying, acquire a silky lustre and form a felted mass. It is difficultly soluble in cold water (1:3500); more readily in hot water and in alcohol (96 per cent.); is insoluble in dilute acids. It dissolves readily in a solution of sodium phosphate, from which solution it is precipitated by hydrochloric acid. Other salts of adenin, as the metaphosphate, we have in the same way. Uric acid is also dissolved by sodium phosphate (WULFF). The water of crystallization is not lost on exposure to air, but is driven off at 100° ; the salt then remains unchanged even at 220° . A cold concentrated aqueous solution of the salt treated with one-tenth its volume of cold concentrated solution of sodium picrate produces a precipitate of short, fine needles, consisting of most of the adenin picrate (five-sevenths). The solubility of the picrate can thus be reduced to as low as 1:13750, and on this

fact is based the quantitative method of BRUHNS. The salt can also be obtained in its characteristic groups by combining cold saturated aqueous adenin solution (1:1086) with pieric acid; with sodium pierate, however, adenin gives no precipitate, since the pierate is soluble in an equivalent quantity of sodium hydrate. Thus is explained KOSSEL's statement that adenin forms an easily soluble compound with pieric acid. Heated on platinum foil it burns slowly and leaves considerable carbon-residue. The very bright yellow color of the salt serves to distinguish it from most of the other pierates, especially guanin pierate. Adenin may be isolated from its pierate by extraction of the hydrochloric acid solution with ether, by precipitation of the ammoniacal solution with silver nitrate, and best, according to KRÜGER, by dissolving the pierate in hot dilute ammonia, and when cold precipitating most of the pieric acid with ammoniacal copper sulphate solution. The filtrate can then be evaporated, dissolved in dilute H_2SO_4 , and the last traces of pieric acid removed with ether.

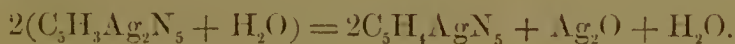
It may be noted that adenin and guanin form difficultly soluble pierates, whereas xanthin and hypoxanthin form relatively easily soluble compounds.

The platinochlorid, $(\text{C}_5\text{H}_5\text{N}_5\text{HCl})_2\text{PtCl}_4$, crystallizes from dilute aqueous solution in small yellow needles. The concentrated aqueous solution of this salt, when boiled for some time, decomposes, with the separation of a clear, yellow powder, which is but slightly soluble in cold water, and has the composition $\text{C}_5\text{H}_5\text{N}_5\text{HCl.PtCl}_4$.

The aurichlorid, on evaporation, yields very characteristic forms. It has been more recently studied by WULF, and found to possess the formula $\text{C}_5\text{H}_5\text{N}_5\text{.(HCl)}_2\text{AuCl}_3 + \text{H}_2\text{O}$. From the hydrochloric acid solution of adenin and gold chlorid, on sufficient concentration, or from dilute solutions by gradual evaporation, it separates in bright, well-formed orange-colored crystals, which may attain a length of 1.2 cm. As pointed out by KOSSEL, this salt is well adapted for the qualitative recognition of adenin, especially in the presence of guanin, which gives no such compound.

Adenin-lead was prepared by KRÜGER by adding a solution of adenin and sodium hydrate to an aqueous solution of lead acetate. It forms lustreless needle-shaped crystals. The composition appears to be $C_5H_3PbN_5$. On friction it becomes strongly electric. Heated with methyl iodid it gives rise to addition products (see page 439).

The silver salt of adenin, $C_5H_4AgN_5$, is formed when silver nitrate is added in molecular proportion to a boiling ammoniacal solution of adenin. On heating this compound for thirteen hours at 130° with methyl iodid no appreciable change results (KRÜGER), although THOISS obtained a compound, presumably a methyl addition product. An excess of silver nitrate produces, in the cold, the compound $C_5H_4Ag_2N_5 + H_2O$, which is converted slowly in the cold, immediately on warming, into the other salt, according to the equation:



Owing to this instability the two compounds are always found together in varying proportion. Both are difficultly soluble in water, and in ammonia even at the boiling-point. The precipitation of adenin by an ammoniacal silver solution is complete, and is therefore available for quantitative estimation. The precipitate of adenin, as well as of other xanthin-bases, is soluble in excess of sodium hyposulphite (KOSSEL).

Adenin silver nitrate, $C_5H_5N_5 \cdot AgNO_3$ ($Ag = 35.4$ per cent.), corresponds to the similar hypoxanthin and guanin salts. It is obtained by dissolving the above silver compound in hot nitric acid; and from this solution, on cooling, it separates in needle-shaped crystals, which are not permanent. This lack of stability, as compared with the permanent hypoxanthin silver nitrate, was first pointed out by KOSSEL, and was thought to be due to loss of nitric acid in washing, and also by heating at 100° . BRUNNS, however, has shown that the acidity of the wash-water is indicated by litmus, but not by methyl-orange, which is not colored red by silver nitrate. It would seem that adenin, as well as hypoxanthin, and possibly xanthin, form

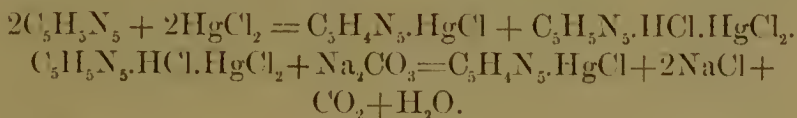
silver compounds containing one and two molecules of silver nitrate; the greater the quantity of silver nitrate used the higher is the per cent. of silver, *i. e.*, the more of the latter compound is formed. These are very unstable, and are decomposed by dilute nitric acid, more so by water, into silver nitrate and the compound containing one molecule of silver nitrate. We have in this behavior an interesting case of mass-action and chemical equilibrium between adenin, silver nitrate, nitric acid, and water. Ammonium hydrate removes the nitric acid from this as easily as from the hypoxanthin compound, and there is formed, according to the composition of the original salt, a varying mixture of $C_5H_4AgN_5$ and $C_5H_3Ag_2N_5 + H_2O$. The solubility in nitric acid is about the same as that of hypoxanthin silver nitrate.

Adenin silver picrate, $C_5H_4AgN_5 \cdot C_6H_2(NO_2)_3OH + H_2O$, is obtained as an amorphous voluminous yellow precipitate when silver nitrate is added to a cold aqueous solution of adenin picrate. If the latter solution is previously raised to the boiling-point, the precipitate then soon becomes crystalline and rapidly subsides. The adenin can thus be almost wholly removed from solution. The crystalline form loses its water of crystallization at 120° , while the amorphous form does not appreciably decrease in weight, and its composition does not appear to be so constant as that of the corresponding hypoxanthin compound. On treatment with ammonium hydrate the picric acid is removed, and adenin-silver, $C_5H_4AgN_5$, is left, stained yellow by traces of picric acid.

Adenin-mercury picrate, $(C_5H_4N_5)_2Hg \cdot 2C_6H_2(NO_2O)_3OH$, can be prepared by treating a hot concentrated aqueous solution of adenin picrate with an excess of sodium picrate, and then with mercuric chlorid. It forms a yellow granular, crystalline precipitate (microscopic needles) which rapidly subsides and increases in quantity as the solution cools. Its composition apparently varies, containing one to two molecules of water, according to the temperature of the solution. One molecule is given off at 100° and the second at 105° – 120° . The latter preparation, then, on exposure to

the air, rapidly absorbs one molecule of water. The object of the sodium picrate in the precipitation is to combine with the hydrochloric acid, which is set free. The precipitate produced by mercuric chlorid in cold adenin picrate solution shows yellow and white granules, and is not homogeneous. BRUNNS considers it to be a mixture of the adenin-mercury picrate and the compound $C_5H_4N_5Hg_2Cl_3$; if sodium picrate is added, however, the pure adenin-mercury picrate forms, since no hydrochloric acid is set free.

Adenin-mercuric chlorid, $C_5H_4N_5HgCl$, is thrown down as a white, finely granular precipitate when a boiling aqueous adenin solution is treated gradually with concentrated mercuric chlorid solution (BRUNNS). On neutralizing the filtrate from this precipitate a second deposit forms. According to KRÜGER, the reaction that takes place is as follows :



Heated with alkyl iodids it does not give rise to substitution-compounds. Free hydrochloric acid is indicated by the reaction with methyl-orange. Treated with ammonium hydrate the chlorine is removed, and there is formed apparently the compound $C_5H_4N_5HgOH$. If dissolved in warm dilute hydrochloric acid and allowed to crystallize, the double salt $C_5H_5N_5.HCl.HgCl_2 + 2H_2O$ separates in long, stellate, silky needles.

Another mercury compound, $C_5H_4N_5Hg_2Cl_3$, is obtained when the precipitation takes place in the cold. The precipitate is white, flocculent, and anhydrous. In this reaction, as above, for each adenin molecule an equivalent of hydrochloric acid is set free. This same body is also produced when an adenin solution is boiled with a large excess of mercuric chlorid and as little hydrochloric acid as possible to effect solution. On cooling small stellate needles separate out, which do not lose their weight at 110° . It can also be obtained by boiling the following compounds with water.

When adenin is boiled with a large excess of mercuric

chlorid and much hydrochloric acid to dissolve completely the precipitate that first forms, there is deposited on cooling a crystalline product, which is variable in its composition, and apparently consists of double salts of adenin and mercuric chlorid, such as $C_5H_5N_3 \cdot HCl \cdot 5HgCl_2$ and $C_5H_5N_3 \cdot HCl \cdot 6HgCl_2$. On boiling with water these rapidly decompose, forming the compound $C_5H_4N_3 \cdot Hg_2Cl_3$. The formation of a double salt, $C_5H_5N_3 \cdot HCl \cdot HgCl_2 + 2H_2O$, is described above.

Adenin-mercury cyanid, $(C_5H_5N_3)_2Hg(CN)_2$, separates as stellate needles and plates when a mixture of hot solutions of adenin and mercuric cyanid are allowed to cool.

An adenin-bismuth iodid, $C_5H_5N_3 \cdot 3H \cdot 2BiI_3 + 2H_2O$, is obtained when an aqueous adenin solution is treated with potassium-bismuth iodid containing free hydriodic acid. The heavy precipitate, which in color resembles carbon monoxid hæmoglobin, consists of microscopic glittering red needles. On contact with much water it partly decomposes, forming light reddish-yellow amorphous floecules, which become darkish brown at 100° .

Brom-adenin. By treating well-dried adenin with excess of dried bromine a dark-red body is obtained which appears to contain six atoms of bromine, $C_5H_5N_3 \cdot Br_6$ (BRUNNEN). On mere exposure to the air, more rapidly on heating at 100° – 120° , it becomes light yellow and decomposes, yielding bromine, brom-adenin, $C_5H_4BrN_3$, and its hydrobromid, $C_5H_4BrN_3 \cdot HBr$. Brom-adenin is white, difficultly soluble in cold water (1:10,000), more readily in hot water, very easily in ammonia and in fixed alkalis. It crystallizes from water or dilute ammonia in stellate needles or very thin plates, which, when dried in air, often assume a silky lustre. The crystals contain a variable amount of water depending on the temperature at which the crystallization takes place. Thus, the crystals may contain almost two molecules of water, whereas when crystallization occurs at above 60° the crystals are anhydrous. It is a rather strong base and forms well-characterized salts which are difficultly soluble in cold water, more easily in the presence of an excess of acid, from which it is

thrown down as a white micro-crystalline precipitate by addition of ammonia. It is also formed from the original dark-red body by treatment with sodium bisulphite, or better by dissolving the body in ammonium hydrate, or, according to KRÜGER, by heating to 130° , then dissolving in sodium hydrate and precipitating with carbonic or acetic acid. It is only difficultly attacked by boiling alcohol or aqueous potash or alcoholic ammonia. The atom of bromine cannot therefore be replaced by an amido- or by a hydroxyl-group. Sodium alcoholate heated with brom-adenin at 145° for hours has no effect.

Brom-adenin is very easily and completely changed to adenin by the action of sodium amalgam in the cold, or by boiling for several hours with zinc-dust. No azulmic acid is formed. It is not affected by iron-dust (BRUNNS). According to KRÜGER, it is affected by heating with concentrated potash at 180° – 190° , and the bromine is not replaceable by radicals as phenol.

Chlor-adenin has not been obtained, since chlorine passed over dry adenin in the cold, or at 100° , or into a boiling chloroformic suspension of adenin is without effect. Phosphorus pentachlorid heated with adenin at 160° – 170° for some hours gave a light brown body of uncertain composition.

The study by BRUNNS of the decomposition of the dark-red body, mentioned above, has shown that it is very probably a hydrobromid of brom-adenin, tetra bromid, $C_5H_4BrN_5 \cdot Br_4 \cdot HBr$. According to KRÜGER, this compound does not always form by the addition of bromine to adenin. Ordinarily the hydrobromid of brom-adenin forms, unless a very large excess of bromine is used. Compounds similar to brom-adenin are formed by hypoxanthin, guanin, xanthin, and caffèin. Azulmic acid reacts with bromine in much the same way as adenin.

The hydrochlorid, sulphate, and nitrate of brom-adenin have been prepared and analyzed by BRUNNS.

Brom-adenin picrate, $C_5H_4BrN_5 \cdot C_6H_2(NO_2)_3OH + H_2O$, resembles that of adenin, but is more voluminous. It is pre-

precipitated under the same conditions as adenin. The solubility in cold water is about the same (1:3220). It is likewise almost completely thrown out of solution by sodium picrate. Under the microscope, however, it can be readily distinguished from adenin picrate, since it does not form distinct crystals, but rather bundles of thin thread-like needles.

The metal-derivatives of brom-adenin are analogous to those of adenin. Thus, ammoniacal silver solution gives rise to a mixture of $C_5H_3AgBrN_5$ and $C_5H_2Ag_2BrN_5 \cdot H_2O$. Silver nitrate produces a gelatinous precipitate which, like the adenin silver nitrate, has an inconstant composition; on careful heating with nitric acid (1.1 sp. g.) it can be obtained in needles which resemble exactly those of the adenin compound. Prolonged boiling with nitric acid results in the separation of silver bromide. Mercuric chlorid, cadmium chlorid, potassium-bismuth iodid, etc., give precipitates with brom-adenin the same as with adenin.

Brom-adenin gives the xanthin-reaction, whereas adenin itself does not. Thus, if evaporated with strong nitric acid on the water-bath to dryness, and the cold yellowish or reddish residue is touched with sodium hydrate, a bluish-violet color forms. With ammonia it is a purple-red; with baryta-water a pure violet.

Dry chlorine gas passed over warm dry brom-adenin has no effect. If, however, the brom-adenin is moist, decomposition and solution result. On evaporation of the solution the residue gives with potassium hydrate an intense violet-red color; baryta produces a bluish-green precipitate.

It is therefore evident from the above reactions with nitric acid and chlorine that brom-adenin is more readily destroyed or oxidized than adenin. Inasmuch as all attempts at obtaining oxidation-products of adenin which would shed light on its constitution failed, the study of the oxidation-products of brom-adenin therefore possessed special interest. KRÜGER has succeeded in oxidizing brom-adenin with hydrochloric acid and potassium chlorate in warm solution, into alloxan, urea, and oxalic acid. A reddish substance which dissolved

in alkalis with a purple-red color was also produced in small amounts. Its alkaline solution gave a dirty-blue precipitate with baryta. The amount of alloxan found was very small; indeed, in one experiment it was entirely absent. Nevertheless, it was sufficient to prove that in adenin, and hence in hypoxanthin, an alloxan-group and probably a urea-residue were present as in uric acid and xanthin.

When adenin is treated with zinc and hydrochloric acid in the cold it forms a difficultly soluble crystalline double salt which has not been obtained in the pure state. This double salt is not obtained by direct treatment of adenin hydrochlorid with zinc chlorid.

One of the hydrogen atoms of adenin is capable of replacement by organic radicals, as seen from the following compounds:

Acetyl-adenin, $C_5H_4N_5.CO.CH_3$, can be obtained by heating the anhydrous base with an excess of acetic anhydride for some time, in an oil-bath, at 130° . It crystallizes in small white scales which dissolve but slightly in cold water and in alcohol; more readily in hot water, in dilute acids and alkalis. Heated to 260° it becomes yellow, but does not melt.

Benzoyl-adenin, $C_5H_4N_5.CO.C_6H_5$, is obtained by the action of benzoic anhydrid, but not of benzoyl chlorid or adenin. It crystallizes from water in long, lustrous, thin needles which sometimes are grouped in bundles and melt at 234° – 235° . It is easily soluble in hot alcohol, from which it recrystallizes on cooling; also in dilute acids and in ammonia. With ammoniacal silver nitrate it gives a precipitate resembling that of adenin, but is more readily soluble in ammonia. This compound is quite stable, since it decomposes very slowly on boiling with hydrochloric acid; on protracted boiling with water it is changed into adenin and benzoic acid.

Mono-benzyl adenin, $(C_5H_4N_5.CH_2.C_6H_5)$, was obtained by THOISS by heating well-dried adenin with benzyl chlorid to boiling (178°) on an oil-bath. It can also be obtained, according to KRÜGER, by heating adenin in a flask with benzyl chlorid in a sulphuric acid bath; also by heating adenin with alcohol, potassium hydrate, and benzyl chlorid under an in-

verted condenser. It crystallizes from alcohol in short, glistening prisms, frequently in small pointed crystals grouped in plate-like aggregations. A ten per cent. alcoholic solution gives reactions with silver nitrate, ammoniacal silver solution, mercuric chlorid, picric acid, and platinum chlorid. Gold chlorid gives no precipitate. The compound forms pure white microscopic crystals and melts at 259° . It is easily soluble in hot water and in hot alcohol; but little in ether. Its solubility in water at 15° is 1 : 2250; in water at 100° is 1 : 320. With acids it forms salts from which alkalis throw down the base. The hydrochlorid, $C_5H_4(C_7H_7)N_5.HCl$, forms fine glassy needles or four-sided glassy prisms with inclined end-surfaces, which are readily soluble in alcohol and in water, but not in ether. The sulphate and nitrate possess similar properties. The sulphate, $(C_5H_4(C_7H_7)N_5)_2.H_2SO_4$, forms glassy long prisms containing five molecules of water, four of which easily pass off at 100° , and the fifth at 110° . Like adenin, it yields a silver compound which is insoluble in ammonia. On reduction with zinc and hydrochloric acid it forms an amorphous red unstable compound. Treated with nitrous acid, benzyl-adenin is reduced to benzyl-hypoxanthin, thus showing that the benzyl-group replaces a hydrogen atom in the group $C_5H_4N_4$, which KOSSEL has called adenyl (see page 452).

Benzyl-adenin pierate, $C_{12}H_{11}N_5.C_6H_2(NO_2)_3OH$, is obtained as fine felted yellow needles, which are fairly soluble in water and in alcohol; insoluble in ether.

Like adenin, the benzyl compound is very resistant to oxidation with potassium permanganate. On treatment with sulphuric acid and chromic acid a part is completely oxidized and the remainder is unchanged. Bromine acts energetically, forming a dark-red sticky mass, which at 120° only gradually gives off a part of the bromine and becomes dark-yellow in color and firmer in consistency. Apparently four atoms of bromine unite with one molecule.

On decomposition with concentrated hydrochloric acid at 180° – 200° it yields glycooll, volatile bases, and a resinous body, $C_{11}H_{12}$, identical with that obtained by Cannizzaro by the action of dehydrating agents on benzyl alcohol (KRÜGER).

Dibenzyl adenin, $C_5H_3(C_7H_7)_2N_5$, is produced, according to KRÜGER, in small amount in the preparation of the mono-benzyl compound. It is best obtained by treating mono-benzyl adenin with benzyl chlorid; or by the action of benzyl chlorid on an alcoholic solution of adenin and potassium hydrate. The free base is obtained by precipitating a solution of the hydrochlorid with ammonium hydrate. It forms fine silky needles which melt at 171° to a yellow fluid. It is easily soluble in ether, very easily in alcohol. In cold water at 13.5° the solubility is 1:13,300; in water at 100° it is 1:1300. A 1 per cent. alcoholic solution gives reactions with silver nitrate, ammoniacal silver solution, mercuric chlorid, copper sulphate, platinum chlorid, and picric acid. Gold chlorid, lead acetate, basic lead acetate, give no precipitate.

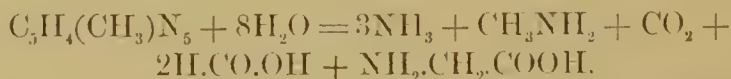
The hydrochlorid, $C_5H_3(C_7H_7)_2N_5.HCl$, crystallizes in fine silky needles, frequently in long prisms with a silky lustre resembling that of caffeine. It is easily soluble in water and in alcohol; insoluble in ether. It is thrown out of water solution, in part, by the addition of hydrochloric acid—a reaction not given by the mono-benzyl compound. The melting-point is at 219° – 220° .

The nitrate, $C_5H_3(C_7H_7)_2N_5.HNO_3$, crystallizes in fine, long glistening needles, which are difficultly soluble in cold dilute nitric acid. It melts at 167° with solution of gas. On decomposition with concentrated hydrochloric acid at 180° – 200° it yields the same products as mono-benzyl adenin (KRÜGER).

Mono-methyl-adenin, $C_5H_4(CH_3)N_5$, was first prepared in a condition of purity by KRÜGER. The methyl-adenin described by THOISS and prepared by the action of methyl iodid on adenin-silver at 100° is probably an addition-product, not methyl-adenin. The introduction of the methyl-group in this way does not take place readily, according to KRÜGER, owing to the formation of addition-products. This addition of methyl iodid can be overcome readily by the presence of sodium alcoholate. This can be done by dissolving adenin in alcoholic sodium hydrate and then adding methyl iodid and allowing to stand for some months, or, better, by warming

under an inverted condenser. It crystallizes from water in anhydrous, long, silky needles or glassy prisms resembling caffeine. On drying in the air the silky lustre disappears. It may crystallize with one and a half molecules of water and does not melt at or below 270° . The gold salt of methyl-adenin crystallizes in fine yellow lustreless needles. The platinochlorid is rather difficultly soluble in cold water and crystallizes in bright six-sided or four-sided rhombic plates. A 1 per cent. aqueous solution of methyl-adenin gives reactions with pierie acid, silver nitrate, ammoniacal silver solution, mercuric chlorid, copper sulphate, and sodium bisulphite. The precipitate by the latter reagent is more soluble than that of adenin. Lead acetate and basic lead acetate give no precipitate. Like adenin, it forms a mono-brom derivative (KRÜGER). The methyl compound of Thoms gave reactions with baryta-water, alcoholic zinc chlorid, mercuric nitrate, and cadmium chlorid, while basic lead acetate was without effect.

On decomposition with concentrated hydrochloric acid at 180° – 200° it yields ammonia, methylamin (distinction from adenin), carbonic acid, formic acid, and glycocoll, according to the equation:



Mono-methyl-adenin methyl iodid, $\text{C}_5\text{H}_4(\text{CH}_3)\text{N}_5.\text{CH}_3\text{I}$, is obtained by the action of methyl iodid on adenin-lead. It crystallizes from alcohol in coarse, glassy small crystals which are easily soluble in alcohol and in water; insoluble in ether. Simple substitution-products do not form readily, but rather addition products.

Ethyl-adenin, $\text{C}_5\text{H}_4(\text{C}_2\text{H}_5)\text{N}_5$, was prepared by KRÜGER according to the principle employed for the preparation of the methyl-derivative. It is easily soluble in water and alcohol, and its aqueous solution gives reactions with silver nitrate, ammoniacal silver nitrate, mercuric chlorid, copper sulphate, and sodium bisulphite, pierie acid, platinum chlorid, gold chlorid. The sulphate crystallizes from concentrated solutions in roundish mass of crystals.

Iso-amyl adenin, $C_5H_4(C_5H_{11})N_5$, was also obtained by KRÜGER by heating adenin with alcohol, sodium hydrate, and iso-amyl iodid. It is easily soluble in alcohol, acetone, chloroform, hot benzol; difficultly soluble in ether and carbon disulphide. The solubility in water at ordinary temperature is 1:1430, and is not increased by sodium or ammonium hydrate. It is easily soluble in acids. It forms large bright irregular plates which melt at 148° – 150° . A 0.7 per cent. aqueous solution reacts with silver nitrate, ammoniacal silver solution, mercuric chlorid, picric acid, and gold chlorid. Platinum chlorid does not give a precipitate.

Nothing definite is known in regard to the physiological action of adenin, except that when fed to dogs it appears to be eliminated as such, in part at least, by the urine.

ADENIN-HYPOXANTHIN, $C_5H_5N_5 + C_5H_4N_4O$. The occurrence of this compound was observed by KOSSEL, but it was isolated and studied for the first time by BRUNN. It can be prepared by cooling a hot aqueous solution of equal parts of the two bases. At first it is obtained as thick, starch-like, semi-transparent masses, which later in part become white and chalky. By spontaneous evaporation of its solution in very dilute ammonia it forms pearly aggregates of very small radially arranged needles, which contain water of crystallization. These effloresce somewhat and lose the water at 100° . The compound is more readily soluble in water than its components, but an exact determination of its solubility is impossible, inasmuch as the separation from hot solutions is not completed for some weeks. Any adenin present can be separated by recrystallization. It forms a distinct crystalline hydrochlorid, which should be borne in mind when examining microscopically for the two bases; but the combination is loose, since addition of gold chlorid brings down the characteristic gold salt of adenin. Ordinarily it does not form salts with sulphuric or nitric acid, but more often is decomposed by these, so that the difficultly soluble adenin crystallizes out. Once, however, BRUNN obtained a sulphate

which differed from the pure adenin and hypoxanthin sulphates; thus is perhaps explained the observation of KOSSEL that adenin sulphate forms crystals belonging to two systems. The compound can be decomposed into its constituents by fractional crystallization of the sulphate or nitrate; but better by forming the picrates, which are very unequally soluble in water. The existence of this compound undoubtedly explains many of the mistakes and discrepancies concerning the properties of hypoxanthin, which it resembles more than adenin, and for the same reason, perhaps, adenin was so often overlooked.

ADENIN-THEOBROMIN, $C_5H_5N_5 \cdot C_7H_8N_4O_2$. This compound resembles the preceding, and was prepared by KRÜGER (1896) from tea-extract. It is easily soluble in hot, more difficultly in cold water. On re-crystallization from water partial decomposition takes place. From aqueous solution picric acid throws down adenin, while theobromin remains in solution. It can be obtained, by crystallizing an aqueous solution of equal molecules of the two bases, as fine, long prisms of marked silky appearance.

HYPOXANTHIN, $C_5H_4N_4O$, sometimes also known as sarkin or sarkin, was discovered by SCHERER (1850) in splenic pulp and in the muscles of the heart, and was named thus because it contains one atom of oxygen less than xanthin. It has since been obtained, usually accompanying adenin and guanin, from nearly all of the animal tissues and organs rich in nucleated cells, *i. e.*, in nuclein. It has been found in blood after death, but not in blood when flowing through the bloodvessels. SALOMON has recently shown it to be a normal constituent of urine, present, however, in an exceedingly minute quantity. In the blood and urine of leucocythæmic patients it occurs in increased quantity owing to the abnormally large number of nucleated white blood-corpuscles in circulation (see page 417). BENGE JONES observed in the urine of a boy, who about three years before showed the symptoms of renal colic, a deposit of characteristic whetstone-like crys-

tals, resembling uric acid, but differing from the latter by dissolving readily on the application of heat, while from hydrochloric acid it crystallized in elongated six-sided plates. These crystals he believed to be those of xanthin, but SCHERER and others consider them to be hypoxanthin. It is therefore quite possible, though very rare, for this base to form a deposit in the urine and to be confounded in shape with uric acid. THUDICHUM has obtained it from the urine of persons sick with liver or kidney diseases. According to JAKSEN, it is present in exudates and transudates with uric acid.

Among other places it has been found in the brain, muscle, serum, marrow of bones, kidney, heart, spleen, liver, peripheral muscles (sarkin of STRECKER); in the spawn of salmon (PICCARD), in the testicles of the bull (SALOMON), in the nuclein of pus and red corpuseles (KOSSEL), in developing eggs, and in putrefaction of albumin (SALOMON). It has also been found in the spores of lycopodium, and in the pollen of various plants, in seed of black pepper, in grass, clover, oats, bran of wheat, larvæ of ants; in the juice of potato (SCHULZE); in certain wines (KAYSER); in the aqueous decoction of yeast of beer (SCHUTZENBERGER); and also in the liquid in which yeast is grown (BECHAMP). DEMANT has shown it to be relatively abundant in the muscles of pigeons in a state of inanition, while in muscles of well-fed pigeons it is said to be entirely absent. SALOMON found hypoxanthin and xanthin in the cotyledons of lupine, as well as in the sprouts of malt, while REINKE and RODEWALD observed these two bases together with guanin in *Æthalinum septicium*—with adenin, xanthin, and theophyllin, it occurs in tea-leaves (KOSSEL); but KRÜGER (1896) showed that it is present in traces, if at all, when the copper method is used. In other words, by the action of nitrous acid, even in presence of urea, adenin is partially changed to hypoxanthin when the silver salts obtained by the old method are treated with nitric acid. BALKE has found it in malt-sprouts by the copper method. In the pollen of the fir (*Pinus sylvestris*) KRESLING found hypoxanthin, xanthin, and quinin, but not adenin. In the seeds of *Randia*

dumetorum VOGTHERR found hypoxanthin and guanin, but no xanthin.

Hypoxanthin has been extracted from the pancreas. Adenin and guanin in the pancreas readily change after death into hypoxanthin and xanthin (INOUE). It seems that hypoxanthin bears a relation to adenin similar to that which we see between glycocoll and glycocollie acid.

Hypoxanthin occurs frequently in plants together with the other members of this group, namely, adenin, guanin, and xanthin. The widely distributed character of these bases is due to the presence of a parent substance, viz., nuelein, the necessary constituent of all cells capable of development, which under the influence of acids, and probably likewise of ferments, decomposes into the above-mentioned bases. They may, therefore, be considered as the first steps in the retrograde metamorphosis of all tissues, since they have their origin in nuelein, an important proteid substance. Recent advances in biological chemistry have shown that the undeveloped eggs of various insects and birds yield much less quantity of xanthin-bodies (hypoxanthin, xanthin, etc.) on treatment with dilute acid than the partially developed eggs (TICHOMIROFF, KOSSEL). This is dependent upon the remarkable fact observed by KOSSEL that the nuelein of undeveloped chicken eggs differs from the nuelein of cell-nuclei and resembles that obtained from milk. For, while the nuelein from the cell-nuclei decomposes into adenin, guanin, hypoxanthin, etc., that from undeveloped eggs and from milk yields no nitrogenous bases on treatment with acids. But as the egg develops, *i. e.*, the nucleated cells increase in number, this latter nuelein is gradually converted or gives way to the ordinary cell-nuelein, and hence it is that the chick embryo yields guanin, hypoxanthin, and possibly adenin.

Unquestionably, the presence of hypoxanthin, etc., in developing cells is due to the presence of the nuelein-molecule, from which it is readily split off. In muscle, however, hypoxanthin and xanthin appear to exist preformed, and bear no relation to nuelein, since they are in the free condition, and

can be extracted from the tissue by water. For an explanation of this peculiar fact, see Adenin, page 417, and Guanin, page 458. For the accidental formation of hypoxanthin from adenin by nitrous acid in the silver method, see page 443.

According to the observations of SALOMON and CHITTENDEN, hypoxanthin is formed by the digestion of blood-fibrin with gastric juice, pancreatic juice, or on heating with water or dilute acids. Egg-albumin under the same conditions does not yield any hypoxanthin, except when treated with pancreatic juice. These observations require repetition, inasmuch as the fibrin used undoubtedly contained nuclein, which, as we now know, readily decomposes under those conditions into its characteristic nitrogenous bases.

When a mixture of guanin, xanthin, and hypoxanthin is allowed to putrefy, the bases decompose and disappear in the order named. Hypoxanthin resists bacterial action the longest, and this corresponds with its behavior to reagents (BACHINSKY). Adenin during putrefaction, in the absence of air, is converted into hypoxanthin, and guanin is correspondingly changed into xanthin (SCHINDLER). An imido-group is, therefore, replaced by oxygen, and probably goes to form urea. This conversion is a very important fact, since the process of putrefaction, as HORPPE-SEYLER has repeatedly pointed out, is analogous to the vital process, and the same chemical change may take place in the animal organs. The same change very probably takes place in the auto digestion of yeast. Its formation under these conditions can be represented thus:



Hypoxanthin can be readily obtained from a number of closely related substances. Thus, carnin, by the action of oxidizing agents, is converted into hypoxanthin. For this reason WEIDEL and SCHÜTZENBERGER regarded hypoxanthin as derived from carnin, but this view is now entirely set aside by our present knowledge of the relation of this base to nuclein.

Again, it can be obtained from adenin (page 426) by the action of nitrous acid. The relation that hypoxanthin bears to uric acid had not been definitely established until KRÜGER showed that the constitution of hypoxanthin was closely connected with that of uric acid and the xanthin-compounds. STRECKER's belief that hypoxanthin by oxidation yields xanthin, and that uric acid by reduction with sodium-amalgam yields first xanthin and then hypoxanthin, was not confirmed by KOSSEL or by FISCHER. In 1895, however, FISCHER succeeded for the first time in demonstrating the relation of uric acid to the xanthin-bases by changing brom-theobromin to a uric acid derivative. Subsequently the reverse change was accomplished by converting a uric acid derivative into theophyllin and this into caffèin.

Hypoxanthin has been hitherto regarded as a step lower than guanin in the series of nitrogenous products of regressive metamorphosis, and consequently was considered as derived from guanin. The investigations of KOSSEL, however, show that it arises not from guanin but from adenin. On the other hand, guanin is to be looked upon as the source of xanthin. It is probable that in the organism it is oxidized as soon as it is set free from the nuclein, forming successively xanthin, uric acid, urea, etc., and the small quantity present in the urine is all that has escaped oxidation. When fed to dogs, it was observed that the amount of hypoxanthin present in the urine decreased, and even became less in amount than before the experiment; but, on the other hand, the amount of xanthin in the urine was found to have increased above the normal. This shows that hypoxanthin in the body is oxidized probably first to xanthin, then into uric acid. According to ROBERT, hypoxanthin is a true muscle stimulant.

The fact that hypoxanthin is so widely distributed in the organism, and in much larger quantities than was formerly supposed, shows that it may constitute, together with the closely related bodies creatin, xanthin, guanin, etc., a part of the antecedents of urea and of uric acid. This view is furthermore strengthened since hypoxanthin is especially

abundant in those organs which are most active in producing metabolic changes in the body, viz., the liver and spleen.

It may be prepared from the urine, according to the method given under paraxanthin; or from extract of meat, or from glandular organs, such as the liver, spleen, etc., by the process on page 419. Nuclein on decomposition with acids yields about one per cent. of this base. It can be determined with adenin indirectly by SCHINDLER'S method (page 421); but better still directly by BRUNNS' pierate method (see page 422). After the adenin has been precipitated by sodium pierate, the determination of hypoxanthin in the filtrate is not difficult if hydrochloric and other acids, the silver salts of which do not quite dissolve in ammonia, are absent. The filtrate from the adenin pierate is rendered slightly alkaline with ammonia and precipitated with silver nitrate at the boiling-point. The slightly yellow-colored precipitate is washed with hot water till the wash-water is colorless; then dried at 120° for from two to three hours, when it has the composition $2C_5H_2Ag_2N_4O + H_2O$. It contains, however, traces of pieric acid and some adenin-silver, and hence the quantity of hypoxanthin calculated from the weight obtained is higher than it really is. BRUNNS, as a correction, subtracts 3.0 mg. from the calculated quantity of hypoxanthin.

A more convenient method than the preceding is to estimate hypoxanthin as hypoxanthin silver pierate. The filtrate from the adenin pierate (page 422) is raised to the boiling-point and silver nitrate solution gradually added. The precipitate is washed with cold water till the wash-water is colorless, then dried at 100° , when its composition is represented by the formula $C_5H_3AgN_4O.C_6H_2(NO_2)_3OH$. The calculated quantity of hypoxanthin here is likewise slightly higher than it should be. BRUNNS deducts 1.0 mg. from the calculated result.

In the presence of hydrochloric acid, etc., the determination of hypoxanthin is somewhat circuitous, since the precipitated silver chlorid must be separated from the hypoxanthin-

compound. The best procedure in this case is to saturate the filtrate from adenin picrate with ammonia and precipitate it completely with silver nitrate. The precipitate is washed with hot water (a thorough washing is not necessary), then it is boiled several times with nitric acid of 1.1 specific gravity. The acid each time is rapidly decanted on to a small filter, and finally the residue washed on the filter with 10 c.c. of the hot acid (total 100 c.c.). To the combined acid filtrate silver nitrate is added, and the whole set aside for twenty-four hours. The precipitate is dried at 100° . The amount of hypoxanthin lost depends upon the quantity of silver chlorid present. The correction to be added is 3.1 mg. (BRUNNS). In Neubauer-Kossel's method the mixed adenin and hypoxanthin silver salts can be decomposed with a little hydrochloric acid and estimated in this way.

Hypoxanthin is a white, colorless, crystalline powder, sometimes in part amorphous; according to BRUNNS, pure hypoxanthin does not form floccules and bunches of microscopic needles, but usually coherent crusts, which consist of roundish, sharp-cornered granules; some resemble quadriatic octahedra. It is soluble in about 300 parts of cold water (STRECKER). The base separates slowly from aqueous solutions, and when pure the solubility, even in the beginning, is less than 1:300. At the end of four days BRUNNS found it to be 1:1880. It is more easily soluble in boiling water (78 parts), and, on cooling, separates in the form of white, crystalline floccules, thus differing from xanthin, which is amorphous. The solubility in cold alcohol is very slight, about 1:1000. It dissolves in acids and alkalis without decomposition, and from solutions in the latter it can be precipitated by passing carbonic acid, or by the addition of acetic acid. The aqueous solution possesses a neutral reaction. The free base can be heated up to 150° without suffering decomposition, but above this temperature it sublimes, and partially decomposes, with evolution of hydrocyanic acid. When heated with potassium hydrate to 200° it yields ammonia and potassium cyanide. Heated with water to 200° it decomposes into carbonic acid, formic acid,

and ammonia, and in this respect it agrees with adenin (page 423). The properties of STRECKER's sarkin agree closely with those of adenin-hypoxanthin; and, inasmuch as the latter has been often described as hypoxanthin, it is very desirable that the properties of hypoxanthin be redetermined.

When evaporated with an oxidizing agent, chlorine-water and nitric acid, the residue is said to give on contact with ammonia vapors a rose-red color (WEIDEL test). KOSSEL, however, has shown that this is due to the presence of xanthin, and that pure hypoxanthin does not give either the murexid test or the xanthin-reaction. According to STRECKER, concentrated nitric acid converts hypoxanthin into a nitro-compound, which in turn, by the action of a reducing agent, is changed into xanthin. This statement has not been confirmed either by FISCHER or by KOSSEL. It does not give a green color with sodium hydrate and chlorid of lime—distinction from xanthin (page 473).

Like adenin, when evaporated with bromine water and nitric acid on a water-bath it gives a residue which with alkalis turns red, whilst nitric acid alone, as given above, has no effect (KOSSEL).

For the behavior of hypoxanthin and other bases to DRECHSEL's reaction, see page 425. With copper sulphate and sodium bisulphite it forms a whiter, more flocculent precipitate than adenin, soluble in 250,000 parts of hot water (KRÜGER). Its solubility and properties are about the same as those of the adenin-compound. 0.5 per cent., and even stronger solutions, are not precipitated in the cold by copper sulphate and sodium hyposulphite. It is, however, precipitated on heating, whereas uric acid is not. It is, therefore, possible to separate uric acid from adenin and hypoxanthin by precipitating the latter two bases in hot solution with copper sulphate and sodium hyposulphite. The method, however, is of little practical value, since uric acid can be readily separated from these two bases with dilute acids. The separation may be useful for guauin and xanthin, which are less soluble in dilute acids, and hence difficult to separate

from uric acid. By effecting the precipitation in cold solution of the two bases, adenin can be separated from hypoxanthin.

With acids it yields crystallizable compounds, and, like the amido-acids, it forms compounds with bases and also with metallic salts, such as silver nitrate and copper acetate.

The hydrochlorid, $C_5H_4N_4O.HCl + H_2O$, crystallizes in needles, and, like the nitrate and sulphate, it is dissociated on contact with water. The crystalline form is characteristic and distinct from that of adenin, as well as adenin-hypoxanthin. The nitrate forms thick prisms or roundish masses readily soluble in water and ammonia. Platinum chlorid forms a yellow crystalline double salt, having the composition $C_5H_4N_4O.HCl.PtCl_4$.

It does not form a difficultly soluble metaphosphate as adenin or guanin (see page 428).

The pierate forms bright yellow prisms easily soluble in hot water, which solution is not affected as that of adenin by sodium pierate. According to WULFF, it possesses the formula $C_5H_4N_4O.C_6H_2(NO_2)_3OH + H_2O$. It is obtained by addition of pieric acid to a solution of adenin, or of sodium pierate to an acid solution of adenin. Depending on the concentration, it precipitates in greater or less length of time. It is difficultly soluble in cold water; easily in alkaline, also in ammonia. The estimation of adenin or guanin by pieric acid in the presence of hypoxanthin is likely to give a high result.

Hypoxanthin-lead can be prepared, according to KRÜGER, by adding a solution of hypoxanthin in sodium hydrate to a solution of lead acetate. It is amorphous.

Hypoxanthin silver, $C_5H_2Ag_2N_4O.H_2O$. All attempts to obtain a compound containing but one atom of silver in the molecule, corresponding to the adenin-compound $C_5H_4AgN_3$, have failed. The above compound was first prepared by STRECKER, and given the formula $C_5H_4N_4O.Ag_2O$; but the former is preferable, since on heating at 120° two and a half molecules of water are lost and $2C_5H_2Ag_2N_4O + H_2O$ ($Ag = 60.2$ per cent.) results. At 140° – 150° it loses again in weight

and becomes gradually gray; on exposure to air it absorbs moisture. In this form hypoxanthin can be estimated quantitatively (see page 446); the presence of sodium picrate does not interfere, but chlorids, etc., do. It is insoluble in hot water. The compound $C_5H_2Ag_2N_4O \cdot 3H_2O$ is obtained in the form of microscopic needles, by treating pure hypoxanthin silver nitrate with excess of aqueous ammonia. On boiling with ammonia-water it is but slightly dissolved, and appears to lose slowly a part of its water of crystallization. As a result of the decomposition one half of the hypoxanthin passes into solution, and can be recovered on boiling with addition of silver nitrate in the crystalline form; or in the cold, as the usual amorphous precipitate, $C_5H_2Ag_2N_4O \cdot H_2O$.

Hypoxanthin silver nitrate, $C_5H_4N_4O \cdot AgNO_3$ ($Ag = 35.29$ per cent.), is the best-known compound; its formula was established by STRECKER. It is obtained by dissolving the above precipitate, produced by addition of silver nitrate to an ammoniacal solution of the base, in hot nitric acid, specific gravity 1.1; on cooling the hypoxanthin silver nitrate crystallizes in the form of tufts of microscopic needles or plates. Heated at 100° – 120° it remains constant in weight; the quantity of silver present, when determined, is always somewhat higher than the theoretical, especially if an excess of silver nitrate is employed in the precipitation. The explanation of this fact is probably that given under adenin, though presence of silver chlorid may partly be the cause. On treatment with ammonia it loses not only nitric acid, but also half of the hypoxanthin, and $C_5H_2Ag_2N_4O \cdot 3H_2O$ forms. The change takes place readily even in the cold, and if during the digestion an excess of silver nitrate is added, the hypoxanthin set free is converted into this compound, which is wholly constant in composition compared with the hypoxanthin silver nitrate. The conversion is quantitative. Very dilute hydrochloric acid, as well as hydrogen sulphide, removes the silver from this compound.

Hypoxanthin-silver picrate, $C_5H_3AgN_4O \cdot C_6H_3(NO_2)_3OH$ ($Ag = 22.88$ per cent.), is gradually formed by adding silver

nitrate to a boiling solution of hypoxanthin picrate. The precipitate is granular and of a lemon-yellow color, and consists of aggregations of fine, short needles. It is slightly soluble in hot, insoluble in cold water. It is, therefore, applicable for a quantitative determination of the base. Aqueous ammonia very readily and completely removes the picric acid from the compound, and the residue is hypoxanthin silver, which is slightly colored yellow by a trace of picric acid; half of the hypoxanthin passes into solution. Nitric acid with difficulty converts it into hypoxanthin silver nitrate.

Hypoxanthin mercuric chlorid, $C_5H_3N_4OHgCl$, is obtained by adding an equivalent quantity of mercuric chlorid to a boiling solution of hypoxanthin. The precipitate, which increases on cooling, is crystalline.

A second compound, $C_5H_3N_4OHg_2Cl_3$, is produced by adding a strong excess of mercuric chlorid, in the cold, to an aqueous solution of hypoxanthin. It forms a heavy granular micro crystalline precipitate, which contains some water of crystallization.

By boiling the preceding compound with just sufficient hydrochloric acid to effect complete solution, there is formed on standing a precipitate of white roundish aggregates of leafy or needle shaped glittering crystals which have the composition $C_5H_4N_4O.HgCl_2 + H_2O$.

The following table of BRUNN illustrates the analogy existing between the mercury-compounds of adenin and hypoxanthin and similar derivatives of ammonium:

AMMONIUM.	ADENIN.	HYPOXANTHIN.
NH_2HgCl	$C_5H_4N_5HgCl$	$C_5H_3N_4OHgCl(: H_2O)$
$NH_2Hg_2Cl_3$	$C_5H_4N_5Hg_2Cl_3$	$C_5H_3N_4OHg_2Cl_3(+ H_2O)$
$(NH_3)_2HgCl_2$	$\left\{ \begin{array}{l} (C_5H_5N_6)_2Hg(CN)_2 \\ (C_5H_5N_6)_2Hg.2C_6H_2(NO_2)_3OH \end{array} \right.$	$C_6H_4N_4OHgCl_2(: H_2O)$

Hypoxanthin, as well as guanin and xanthin, forms readily soluble compounds with fixed alkalis. From these solutions the alkali compounds tend to crystallize on gradual evaporation, in rosettes or bundles of needles—difference from heteroxanthin and paraxanthin (SALOMON). The alkali solutions of the xanthin-bases are precipitated by carbonic acid and

behave with acid salts, bicarbonates, and ammonium salts the same as the heteroxanthin-sodium compound.

According to BRUNNS, hypoxanthin and uric acid are unaffected by the action of dry bromine, even at 100° , but KRÜGER has shown this statement to be incorrect. Bromine has no action on hypoxanthin at ordinary temperatures, but at 100° and as high as 150° the latter is changed quantitatively into brom-hypoxanthin. A dark-red crystalline mass is obtained which contains six atoms of bromine to one molecule of hypoxanthin. It is a tetra-bromid of brom-hypoxanthin hydrobromid, $C_5H_3BrN_4O \cdot 4HBr \cdot Br_2$, analogous to the similar compound of adenin. It loses bromine slowly in the cold, rapidly at 120° , and brom-hypoxanthin hydrobromid remains, $C_5H_3BrN_4O \cdot 4HBr$. From this salt the free base can be obtained after conversion into the sodium compound. For this purpose the solution of the salt is treated with sodium hydrate or carbonic acid, or saturated direct with sodium carbonate, then concentrated to crystallization.

Brom-hypoxanthin, $C_5H_3BrN_4O + 2H_2O$. This can be prepared as just described, or by the action of nitrous acid on brom-adenin at 70° . It forms a heavy powder of small, coarse crystals; may form spherical groups of long, hair-like needles containing $1\frac{1}{2}$ molecules of water. It is diffcultly soluble in water; easily soluble in acids and alkalis. The aqueous solution reacts strongly acid. It has the properties of a base and an acid, behaving with alkalis and alkali carbonates the same as uric acid, setting free carbonic acid from the latter.

An aqueous solution of brom-hypoxanthin reacts with silver nitrate, ammoniacal silver solution, mercuric chlorid, copper sulphate, and sodium bisulphite. In acid solution it is precipitated by tannic acid, phosphotungstic acid, phosphomolybdic acid, by basic lead acetate; and is not precipitated by lead acetate or by baryta-water.

On heating brom hypoxanthin with sodium carbonate, or on passing carbonic acid into a solution of the base in sodium hydrate, the sodium compound of brom-hypoxanthin forms,

$C_5H_2NaBrN_4O + 2H_2O$. It is easily soluble in hot water; rather difficultly in cold water. The solution has an alkaline reaction. The free base can be obtained by adding the calculated quantity of acid. The corresponding barium-compound is obtained by passing carbonic acid into a solution of brom-hypoxanthin in barium hydrate. On concentration it crystallizes in fine white needles, and has the composition $(C_5H_2BrN_4O)_2Ba$. The lead-compound forms on the addition of lead acetate to a solution of the base in sodium hydrate as an amorphous precipitate.

The bromine in brom-hypoxanthin is held as firmly as that in brom adenin. Thus, it is not affected by heating with alcoholic potash for three hours.

On decomposition of brom-hypoxanthin with hydrochloric acid and potassium chlorate KRÜGER obtained alloxan and urea. The yield of alloxan is not greater than that from adenin, and much less than that from xanthin, due undoubtedly to the alloxan-nucleus splitting up into simpler bodies because of a different arrangement of bonds.

Benzyl-hypoxanthin, $C_5H_3N_4O.CHI_2.C_6H_5$, was obtained by THOISS by the action of nitrous acid on benzyl-adenin. It forms a white crystalline mass which under the microscope consists of thin plates. It is easily soluble in hot water, dilute alcohol, and in acetic ether; insoluble in ether and chloroform. It melts at 280° . It appears, as KOSSEL has pointed out, that adenin and hypoxanthin contain a group, $C_5H_4N_4$, which he named *adenyl*. The formation of the benzyl derivatives of these two bases shows that the hydrogen atom which is replaced occurs in the adenyl- and not in the imido group. According to this view, adenin is to be considered as adenyl-imid ($C_5H_4N_4.NH$) and hypoxanthin as adenyl oxid ($C_5H_4N_4O$).

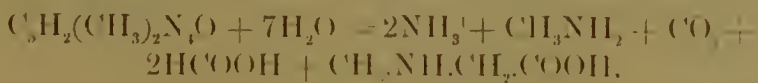
Dimethyl-hypoxanthin, $C_5H_2(CH_3)_2N_4O$. This compound was prepared by BRUNNS, in the same way as methyl-adenin, by heating hypoxanthin with alcohol, sodium alcoholate, and methyl iodid. A compound of dimethyl hypoxanthin with sodium iodid, $C_5H_2(CH_3)_2N_4O.NaI + 3H_2O$, formed first, which

crystallized from alcohol forms prismatic crystals, easily soluble in water and in hot alcohol, insoluble in ether. Other bases of the uric acid group are not known to form similar compounds. Even caffeine, which resembles dimethyl-hypoxanthin, does not enter into such a combination.

The free base is obtained by treatment of this compound with freshly precipitated silver oxide. From chloroform it crystallizes in fine silky needles, containing three molecules of water of crystallization. From alcohol it crystallizes in groups of small pointed anhydrous crystals. It is easily soluble in water and chloroform; less so in alcohol. A strong solution of the base reacts with silver nitrate, with nitric acid, and silver nitrate, with copper sulphate and sodium bisulphite (in the cold, not on warming). Copper sulphate and sodium thiosulphate do not give a precipitate. A one per cent. solution of the base gives precipitates with mercuric chlorid, platinum chlorid, and gold chlorid, but not with lead acetate, basic lead acetate, picric acid, and ammoniacal silver solution.

The existence of this base demonstrates the presence in hypoxanthin, and hence in adenin, of two imido groups. On account of the solubility of this base in water and chloroform; the fact that it is not precipitated by ammoniacal silver solution, which precipitates all bases of the uric acid group containing a replaceable imido-group; also on account of its behavior with copper sulphate and mercuric chlorid, KRÜGER concluded that hypoxanthin contains only two imido-groups capable of substitution. In addition to these adenin contains a third amido-group, which is not replaceable, and which with nitrous acid is replaced by oxygen.

On decomposition with concentrated hydrochloric acid at 180°–200° it yields one molecule of methylamin, two molecules of ammonia and sarkosin, according to the equation:



Diethyl-hypoxanthin ethyl iodid, $\text{C}_5\text{H}_2(\text{C}_2\text{H}_5)_2\text{N}_4\text{O} \cdot \text{C}_2\text{H}_5\text{I}$. This compound was also prepared by KRÜGER, by heating

hypoxanthin-lead with ethyl iodid. It crystallizes from alcohol in beautiful four-sided glistening prisms, which are easily soluble in water and in hot alcohol; insoluble in ether. The existence of this body, as well as the preceding, shows that hypoxanthin, and hence adenin, contains two imido-groups which can be substituted by alkyl radicals. Under the same conditions adenin always forms the mono-substituted compound.

Iso-amyl hypoxanthin, $C_5H_3(C_5H_{11})N_4O$, was likewise prepared by KRÜGER by heating hypoxanthin in the presence of sodium hydrate and alcohol with amyl iodid. It forms six-sided rhombic plates, and is easily soluble in chloroform, difficultly in cold water, insoluble in ether.

Di-iso-amyl hypoxanthin, $C_5H_2(C_5H_{11})_2N_4O$, is formed in small amount at the same time as the preceding. It yields a hydrochlorid crystallizing in small needles. The base is set free from solutions of salt as an oil, which on cooling becomes crystalline. The formation of these two compounds is analogous to the benzyl substitutions in adenin.

Ethyl chloro-carbonate, acting on hypoxanthin in the presence of sodium hydrate, produces a precipitate, which, recrystallized from hot water, forms elongated sharp-angled plates which melt at 185° – 190° . It is insoluble or difficultly soluble in cold or hot alcohol, in ether, and in cold water; easily soluble in hot water, in sodium hydrate, and in hydrochloric acid. Its formula corresponds to $C_5H_3N_4O.CO.C_2H_5$. It is, therefore, considered by KOSSEL to be a urethan of hypoxanthin.

Phosphomolybdic acid precipitates hypoxanthin from acid solution, and in general it gives the ordinary alkaloidal reactions.

It is not precipitated by ammoniacal basic lead acetate. Copper acetate does not precipitate it in the cold, but does on boiling. This fact has been made use of in the isolation of hypoxanthin. Mercuric chlorid, as well as mercuric nitrate, produces a flocculent precipitate.

Altogether, in its behavior to reagents it resembles xanthin

to a very considerable degree. The two can be separated, however, by the different solubilities of the hydrochlorids in water, and more especially of the silver salt in nitric acid.

Physiological Action.—25–100 mg. begin to act on frogs in from six to twenty-four hours, and produce increased reflex excitability and convulsive attacks; 5–100 mg. are fatal (FILEHNE). When injected subcutaneously into hepatotomized geese or chickens, or when fed to chickens, a corresponding increase in uric acid secretion is observed (v. MACH). This conversion is analogous to that observed by STADTHAGEN in the case of guanin (page 459), and shows that in the xanthin-bodies we may have antecedents of uric acid apart from the synthesis of the latter from ammonia in the liver, or from the direct decomposition of nucleins. The process by which this change is effected is undoubtedly one of oxidation.

GUANIN, $C_5H_5N_5O$, was discovered, in 1844, by UNGER, as a constituent of guano, in which it is present in varying quantities according to the region from which the guano comes. Thus the Peruvian guano is reported as containing the largest proportion of this base, and on that account this variety is employed when it is desired to prepare guanin. Since its discovery by UNGER it has been met with in a very large number of tissues, both animal and vegetable; in the liver, pancreas, lungs, retina, in the thymus gland of the calf, and in the testicle-substance of the bull; in the scales of the bleak, and in the swimming-bladder of fish, as well as in the excrements of birds, of insects, as the garden spider, in which it occurs with a small quantity of uric acid (WEINMANN), and is to be regarded as a decomposition-product of proteids formed in the tissues of the spider. It is also found in the spawn and testicle of salmon, and SCHULZE and others have shown it to be present in the young leaves of the plane-tree, of vine, etc., also in grass, clover, oats, as well as in the pollen of various plants. KRESLING has found it in the pollen of the fir with hypoxanthin and xanthin.

Guanin and hypoxanthin, but no xanthin, are present in the seeds of *Randia dumetorum* (VOGTHERR). SCHUTZENBERGER has isolated it, together with hypoxanthin, xanthin, and carnin, from yeast which had been allowed to stand in contact with water at near the body-temperature. Pathologically, it occurs in the muscles, ligaments, and joints of swine suffering from the disease known as guanin gout. Normally, guanin, like adenin, is present in muscle-tissue only in traces. It has never been found in the urine, though xanthin has been mistaken for guanin by some. In some cases of exudates and transudates guanin is present in considerable amount (JAKSCH).

As to the origin of this substance in the organism very little has been known up to within a few years, except so far as it has been shown to be, together with other members of this group, a transitory product in the retrograde metamorphosis of nitrogenous foods and tissues. In the case of the lower animals it is evidently the end-product of all change, inasmuch as it is excreted as such. Our knowledge as to the immediate origin of this and the other allied bases has lately been extended by the brilliant researches of KOSSEL and his pupils on the decomposition-products of nuclein, in which he has shown that this essential constituent of all nucleated cells, whether animal or vegetable, decomposes under the action of water or dilute acids into adenin, guanin, hypoxanthin, and xanthin. We know that the first two bases are readily converted by the action of nitrous acid into the other two; that is to say, a NH group in these bases is replaced by an atom of O—a change which it is not at all unlikely takes place in the tissues, perhaps in every cell-nucleus. That such a change is quite probable is shown by the putrefaction-experiments of SCHINDLER, whereby adenin and guanin were converted respectively into hypoxanthin and xanthin. If this explanation is correct, then adenin and guanin are transition products between the complex proteid molecule on the one hand, and hypoxanthin and xanthin on the other. These two, in turn, form the connecting link to the last step

in the regressive metamorphosis of the nitrogenous elements of the tissues, viz., the formation of uric acid and urea. We can thus trace a succession of steps from the complex nuclein-molecule, which is apparently indispensable to the functional activity of all reproductable cells, to the physiologically waste-products urea and uric acid.

SCHULZE and BOSSHARD recently (1886) found in young vetch, clover, ergot, etc, a new base, to which they have given the name vernin. It has the formula $C_{16}H_{20}N_8O_8$, and is of especial interest at this point, since on heating with hydrochloric acid it apparently yields guanin. We have, therefore, at least two well-defined sources of guanin, the nucleins and vernin.

Neither adenin nor guanin occurs in normal muscle further than in mere traces, a fact which can only be explained on the ground that the muscle-tissue is poor in nucleated cells, and hence in nuclein. Just as the muscle-cell has become morphologically differentiated from the typical cell, it may be looked upon also as having undergone a concomitant chemical differentiation, inasmuch as we no longer find the phosphoric acid, xanthin, and hypoxanthin in the same chemical combination as they occur in the original cell. The phosphoric acid, instead of existing as a part of an organic compound, is present in the muscle-tissue as a salt; similarly the hypoxanthin and xanthin occur in the free condition, extractable by water, and no longer in combination with other groups of atoms constituting a part of a more complex molecule—nuclein.

Guanin and creatin apparently mutually replace one another. Thus, in the muscle, as just stated, guanin occurs only in traces, whereas creatin is especially abundant. This may find its explanation in the fact that both are substituted-guanidins. Creatin is regarded by HORPE-SEYLER as an intermediate product in the formation of urea, and a similar rôle probably belongs to guanin. From STADTHAGEN'S experiments on dogs we know that guanin ingested produces an increase in the amount of uric acid and urea excreted, and the same is also true of the nuclein derived from yeast. These

results have led him to the conclusion that in mammals uric acid is a direct, or more or less altered cleavage-product of proteids, notwithstanding the fact that in birds it is the result of synthesis in the liver.

In the decomposition of nuclein-containing substances, such as yeast, liver, spleen, etc., by dilute acids, neither adenin nor guanin is found alone, but they are always accompanied by hypoxanthin, and usually by a very small quantity of xanthin.

Guanin may be readily prepared from Peruvian guano by boiling it repeatedly with milk of lime until the liquid becomes colorless. The residue, consisting largely of uric acid and guanin, is boiled with a solution of sodium carbonate, filtered, and the filtrate, after the addition of sodium acetate, is strongly acidulated with hydrochloric acid. This precipitates the guanin, together with some uric acid. The precipitate is dissolved in boiling hydrochloric acid, and the guanin then thrown out of solution by the addition of ammonium hydrate.

A more convenient method of isolation of guanin from Peruvian guano is that of WULFE. The guano is boiled with about 5 per cent. sulphuric acid for 4-6 hours, then cooled, and at once filtered. The filtrate is rendered alkaline with sodium hydrate and again filtered. This filtrate, containing guanin and a little uric acid, is now precipitated with ammoniacal silver solution. The voluminous precipitate, after standing twelve hours, is transferred to a thick plaited filter, and washed first with cold then with hot water. While still moist the precipitate is removed from the filter and introduced gradually into hot, dilute hydrochloric acid. The silver chlorid is filtered off, and the filtrate digested on a water-bath with animal charcoal. The clear solution is then saturated with ammonium hydrate to precipitate the guanin. In order to destroy the traces of uric acid which accompany the guanin the precipitate is dissolved, together with a small amount of urea, in boiling 20 per cent. nitric acid, then set aside to crystallize. The nitrate of guanin is now dissolved

in dilute sodium hydrate, and reprecipitated by addition of ammonium chlorid, thus removing any traces of xanthin that may be present.

Inasmuch as the guanin is present in guano in combination partly with calcium, partly as a nuclein-like body, it is not all set free by a single boiling with dilute acid. The extraction should, therefore, be repeated until it ceases to be given off.

Guanin is also obtained in the decomposition of nuclein with dilute acids, and can, therefore, be prepared from such cellular organs as the spleen, pancreas, etc. It should be noted here that in the decomposition of the mixed silver-compounds with hydrogen sulphid or ammonium sulphid (SCHINDLER) the guanin, often only in part passes into solution with adenin and hypoxanthin, and the remainder is held back in the silver sulphid precipitate. The latter should, therefore, be boiled with dilute hydrochloric acid, and on saturating the filtrate with ammonia the guanin after a while separates. That portion of the guanin which did pass into solution with the other two bases is separated from them by digestion with ammonia on a water-bath. The two portions are then combined, transferred to a filter previously dried at 110° , and weighed, washed well with ammonia, then dried and weighed.

Owing to the slight solubility of guanin picrate it has been proposed by WULF as a means for the estimation of guanin. For this purpose the neutral or acid guanin solution is precipitated while warm, with a sufficient amount of cold saturated picric acid solution. After standing twenty-four hours the solution is filtered through a hardened filter, and the precipitate well drained. It is then washed with one per cent. picric acid, and allowed to drain, after which it is placed between two watch-glasses, and dried by slowly raising the temperature, finally for one and a half hours at 110° . A deduction is made for the free picric acid by determining the amount of water in the precipitate, from the difference in the weights, before and after drying. Allowance should be made for one

molecule of water of crystallization that is driven off. A further correction for the solubility of the guanin salt should be made by adding 0.0035 for each 100 c.c. of the combined filtrate and wash-water. The results thus obtained are quite satisfactory. BRUHNS has employed the picrate of adenin in the estimation of adenin. Xanthin and hypoxanthin were supposed to yield soluble compounds with picric acid, so that either adenin or guanin, or both, could be separated from these bases in this way. WULFF, however, has shown that when guanin is precipitated by picric acid in the presence of hypoxanthin some of the latter is also precipitated, so that it is not possible to separate the two bases in this way. The same is true of adenin and hypoxanthin if the picrate is not filtered off until after some hours.

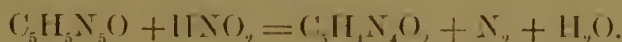
In the separation of adenin and hypoxanthin from guanin by heating with ammonium hydrate, some guanin is dissolved, so that the filtrate cannot be used with accuracy for the separation of adenin from hypoxanthin by BRUHNS' method. WULFF has endeavored to replace the ammonia with metaphosphoric acid. Guanin is precipitated from feebly acid solutions by metaphosphoric acid almost completely. The precipitate filtered off, washed with cold water, dried at 110° , and weighed as $C_5H_5N_5O.HPO_3 + \frac{1}{2}H_2O$, gives usually slightly low results, owing to the difficulty of washing the precipitate, and the fact that the amount of water retained in drying varies through the partial conversion into orthophosphate. Where more accurate results are desired the precipitate can be transferred to a Kjeldahl flask, and the nitrogen determined. The guanin can then be calculated from the amount of nitrogen found.

Although adenin is precipitated slowly by metaphosphoric acid, it does not interfere with the separation of guanin, since it is soluble in large excess of reagent. Hypoxanthin does not give rise to a difficultly soluble metaphosphate, hence does not interfere with the precipitation of guanin. In the filtrate from guanin metaphosphate the hypoxanthin can be determined directly by BRUHNS' hypoxanthin silver

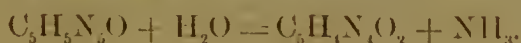
piecate method, though on account of the excess of metaphosphoric acid in the filtrate it is not to be recommended. It would be better to precipitate the filtrate with ammoniacal silver solution, to decompose the silver salts with dilute hydrochloric acid, and then in the filtrate to separate, according to BRUHNS, the adenin from the hypoxanthin. The precipitation of guanin in the presence of adenin should be carried out in very dilute solutions, and, as stated, an excess of reagents should be employed. The method possesses decided advantages over the ammonia method of separation, owing to the solubility of guanin in ammonia, especially when hot. According to WULFF, 100 c.c. of a five per cent solution of ammonia dissolves in the cold 0.01 g. of guanin. For the volumetric estimation of guanin by the copper method of BALKE, see page 477.

The free base forms a white, amorphous powder, insoluble in water, alcohol, ether, and ammonium hydrate; easily soluble in mineral acids, fixed alkalis, and in excess of concentrated ammonium hydrate. It can be heated to above 200° without undergoing decomposition. When evaporated with strong nitric acid it gives a yellow residue, and this on the addition of sodium hydrate assumes a red color, which on heating becomes purple, then indigo-blue; on cooling it returns to a yellow, passing through purple and reddish-yellow shades, due, according to v. BRÜCKE, to absorption of water. This is the so-called xanthin-reaction, and is supposed to be due to the formation of xanthin and a nitro-product. It is given best by guanin, then by xanthin, and is not given by either hypoxanthin or adenin.

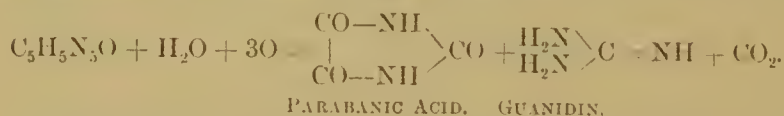
Nitrous acid converts it directly into xanthin, thus:



This reaction is identical with that of adenin, whereby hypoxanthin is formed (see page 426). By putrefaction in the absence of air it forms xanthin (SCHINDLER). The change can be represented by the equation:



On oxidation with potassium permanganate it yields urea, oxalic acid, and oxy-guanin. By hydrochloric acid and potassium chlorate it is oxidized to carbonic acid, guanidin, and parabanic acid, according to the equation :



This decomposition by STRECKER was repeated and confirmed by FISCHER, who was unable to obtain alloxan and urea, as in the case of xanthin, but instead parabanic acid and guanidin.

According to STRECKER, a small amount of xanthin is formed in this reaction, and it is quite possible that this base is also formed on oxidation with nitric acid, especially if it contains nitrous acid.

On decomposition with concentrated hydrochloric acid at 180° – 200° WULF obtained similar cleavage-products as those of xanthin, namely, ammonia, glycocoll, carbonic acid, and formic acid. The reaction is as follows :



Guanin combines with acids, bases, and salts. It is a very feeble base as seen from the fact that some of its salts, as the hydrochlorid, bichromate, etc., readily dissociate on contact with water, especially at higher temperatures. On the other hand, adenin is a much stronger base. It unites with bases to form crystalline compounds; and with one or two equivalents of acid to form crystallizable salts. Thus, with hydrochloric acid it forms the two salts, $\text{C}_5\text{H}_5\text{N}_5\text{O}.\text{(HCl)}_2$ and $\text{C}_5\text{H}_5\text{N}_5\text{O}.\text{HCl} + \text{H}_2\text{O}$. Similar combinations can be obtained with nitric acid. The sulphate, $(\text{C}_5\text{H}_5\text{N}_5\text{O})_2\text{H}_2\text{SO}_4$, crystallizes in long needles, and, like the other salts, is decomposable by water. The nitrate, oxalate, and tartrate are also known.

The platinochlorid, $(\text{C}_5\text{H}_5\text{N}_5\text{O}.\text{HCl})_2\text{PtCl}_4 + 2\text{H}_2\text{O}$, is readily obtained in a crystalline condition. The silver compound is soluble in hot nitric acid, and on cooling recrystallizes in fine, needle-shaped crystals, having the composition

$C_5H_5N_5O.AgNO_3$. For its behavior to DRECHSEL'S reaction, precipitation with copper solution in the presence of reducing substances, see page 425. The compound forms as a white flocculent precipitate, which soon turns greenish and tends to dissociate on contact with water (BALKE). It probably has the formula $C_5H_5N_5O.Cu_2O$. As it has more imido-groups than adenin and hypoxanthin, it is probable that its solubility is less than that of the copper compound of these bases.

The solutions of the hydrochlorid are precipitated by mercuric chlorid and nitrate, potassium chromate, potassium ferrieyanide, and by picric acid. Basic lead acetate gives a precipitate only on addition of ammonium hydrate.

Guanin bichromate, $(C_5H_5N_5O)_2.H_2Cr_2O_7$, is in composition analogous to that of adenin, and was obtained by WULF by adding potassium bichromate to a hydrochloric acid solution of guanin. It appears as well-formed, bright, orange-colored, elongated, four-sided prisms, with truncated ends. On contact with water it readily dissociates, especially at 100° . When heated above 100° it gives off water, whereas adenin, which is a stronger base, does not dissociate on contact with water, and is permanent at above 100° .

Guanin metaphosphate, $C_5H_5N_5O.HPO_3 + H_2O$, has also been studied by WULF. It is characterized by extremely slight solubility in water and in dilute acids. POHL first observed that sodium metaphosphate gave with guanin hydrochlorid a precipitate, insoluble in excess of acids, but soluble easily in alkalis. LIEBERMANN obtained a precipitate by adding metaphosphoric acid to a solution of guanin in sodium hydrate. The same precipitate, according to WULF, forms when an acid solution of guanin is treated with metaphosphoric acid. Contrary to LIEBERMANN, the salt has a definite composition, and is not prone to dissociation as is the case with other compounds of guanin. The precipitation of guanin is so complete that in the filtrate picric acid gives no reaction, and silver nitrate produces only a slight flocculent precipitate. Inorganic salts, as magnesium sulphate, may prevent or retard the precipitation. It forms an amorphous,

porcelain-like mass, which is ignited with difficulty. Dried at 120° it still retains one-half molecule of water. In the presence of water at high temperature, as in drying, a part of the compound is converted apparently into the orthophosphate, so that very accurate quantitative results are not possible.

LIEBERMANN'S view that nuclein is a metaphosphate of albumin containing mechanical admixtures of metaphosphates of xanthin-bases has been shown to be wrong. As additional evidence is the fact that guaniu metaphosphate is very difficultly soluble in ammonium hydrate, while nuclein is extremely soluble in dilute ammonia.

Guanin ferricyanid, $(C_5H_5N_5O)_4 \cdot H_3Fe(CN)_6 + 8H_2O$. This compound separates slowly, when potassium ferricyanid is added to a hydrochlorid guanin, as small, bright, brownish-yellow four- or six-sided prisms. At 100° it slowly loses weight, and heating for several hours at 120° – 130° is necessary to expel all the water. The composition of this salt is noteworthy, for one molecule of acid combines with four of guanin. The trivalent ferricyanic acid does not always exist as such in combination, as in the case of the salt of guanin. On the other hand, as pointed out by WULF, guanin, though usually a monacid base, may unite with different proportions of acid. Thus four nitrates are known, while in the oxalate and tartrate three molecules of guanin unite with two molecules of acid.

Guanin also forms a compound with ferrocyanic acid, which appears as almost colorless needles.

Guanin nitroferrocyanid, $(C_5H_5N_5O)_2 \cdot H_2NOFe(CN)_5 + 1\frac{1}{2}H_2O$. This is likewise obtained by adding sodium nitroprussid to a solution of guanin hydrochlorid. It forms large, glistening, bright brick-red, four-sided prisms with pointed ends.

Potassium-bismuth iodid produces in even very dilute solution of the salts of guanin a precipitate which consists of fine, rather long red needles. When dry it forms a loose, deep-red mass. On heating even below 100° water is given off and

the color changes to a dark violet. It dissociates readily on contact with water. The formula as determined by WULFF is $C_5H_5N_5.OH.2BiI_3 + 2H_2O$.

Guanin picrate, $C_5H_5N_5O.C_6H_2(NO_2)_3OH + H_2O$. The reaction with picric acid (CAPRANICA) is said to be very characteristic, and a means of distinguishing this base from xanthin and hypoxanthin. It is best obtained by adding a cold, saturated solution of picric acid or of sodium picrate to the warm acidulated solution of guanin, when a light, crystalline precipitate forms. Under the microscope it appears in pencil-shaped, fern-like tufts of fine, orange-yellow needles, rarely in bunches of large needles. Adenin picrate has a lighter color. This compound is characterized by its crystalline form and its extreme insolubility in cold water and in dilute acids. Guanin solutions, 1 : 30,000, are still precipitated by picric acid, though after some time. When dry it has a gold-yellow color, felt-like consistency, and silky lustre. On heating it becomes almost orange-red, and on cooling the original color returns. At 110° it loses water of crystallization, the silky lustre, and becomes light yellow; at 190° it begins to decompose. It dissolves easily on warming in fixed alkalis and carbonates. A solution of guanin-sodium is therefore precipitated only by excess of picric acid. It is rather easily soluble in warm dilute acids; difficultly in cold acids. It is dissociated by water, alcohol, and ammonia, especially when warmed (WULFF).

Guanin silver picrate, $C_5H_4AgN_5O.C_6H_2(NO_2)_3OH + 1\frac{1}{2}H_2O$, is thrown down from a boiling solution of a guanin salt, treated with excess of picric acid, by silver nitrate as a voluminous, lemon yellow amorphous precipitate. It is very difficultly soluble in hot water, almost insoluble in cold water. It tends to become dissociated on contact with water—picric acid being removed. Ammonia removes the picric acid easily and completely, leaving guanin-silver (WULFF).

Guanin sodium dissociates on contact with water.

Brom-guanin, $C_5H_4BrN_5O$, was prepared by FISCHER and

Reese in 1883, and is analogous to brom-caffen, adenin, and hypoxanthin. The hydrochlorid dissociates at ordinary temperature. By the action of nitrous acid it is changed into brom-xanthin just as brom-adenin is converted into brom-hypoxanthin.

Acetyl-guanin, $C_5H_4N_5O.CO.CH_3$, was prepared by WULF by heating dry powdered guanin with acetic anhydrid. It forms small, colorless, silky needles which are very difficultly soluble in cold water—in about 4000 parts; more difficultly in cold alcohol and almost insoluble in ether. It is soluble in about 150 parts of boiling water; less easily in hot alcohol. It is easily soluble in dilute acids, alkalis, and ammonia, especially on warming. On heating with acids and alkalis it is completely saponified. From a solution in cold dilute sodium hydrate it is precipitated unchanged by carbonic acid. It is likewise unaffected by boiling water. At 260° it is apparently unchanged.

Propionyl-guanin, $C_5H_4N_5O.CO.CH_2.CH_3$, was also prepared by WULF by heating dried guanin with propionic anhydrid. Only a small part of the guanin enters into combination. It forms peculiar crystals, which, under the microscope, appear as rather long plates or scales with frequently notched edges. The precipitate is very voluminous, and when dried forms a light, felt-like, white mass having a mother-of-pearl lustre. Its properties correspond to the acetyl compound. It is likewise apparently unaffected at 260° .

Benzoyl-guanin, $C_5H_4N_5O.CO.C_6H_5$. Benzoic anhydrid reacts even less energetically than propionic anhydrid on guanin, and only a very small quantity of the esther forms. It appears as small round masses, which consist of fine stellate or bunched needles. It is difficultly soluble in hot water and alcohol, insoluble in ether. At very high temperature decomposes with brown coloration. It is rather resistant to the action of boiling water, but is readily saponified by hot dilute acids. Attempts to obtain this compound by the action of benzoyl chlorid on guanin or on guanin-silver failed.

Alkyl-derivatives of guanin. FISCHER and REESE at-

tempted unsuccessfully to prepare alkyl-derivatives of guanin by the action of methyl iodid on the lead and silver compounds of guanin. WULFF endeavored to prepare benzyl-guanin in the same way that THOISS made benzyl-adenin, but failed. Even heating benzyl chlorid with guanin in the presence of sodium hydrate failed. Attempts to prepare a dimethyl-derivative were likewise resultless.

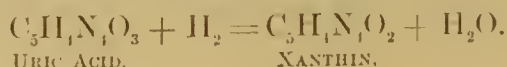
Ethyl-guanin, $C_5H_4N_5O.C_2H_5$, was obtained by WULFF by heating guanin with ethyl iodid in the presence of sodium hydrate and alcohol. It forms small needle-shaped crystals. When dry it yields a light, dry mass. It is difficultly soluble in water, very difficultly in alcohol, easily in mineral acids. In general it gives the same reactions as guanin. Thus, it yields a silver compound difficultly soluble in ammonium hydrate; a finely crystalline picrate, etc. The boiling-hot aqueous solution is precipitated by gold chlorid. Apparently it is not altered by heating at 280° .

Physiologically guanin, like uric acid, is inert (FILEHNE).

XANTHIN, $C_5H_4N_4O_2$, is also very widely distributed in the organism, and has been met with in almost all the tissues and liquids of the animal economy. Together with hypoxanthin, guanin, and possibly adenin, it occurs in many plants, among which may be mentioned lupin, aethalium, sprouts of malt, tea-leaves (BAGINSKY), auto-digestion of yeast, gourd seeds, soja beans, etc., in sprouts of *Cicer arietinum* (BELSUNG). Xanthin bases are found in sprouts of lupin and of the gourd; with hypoxanthin and guanin it occurs in the fir, *Pinus sylvestris*. It was first discovered by MARCET (1819) in an urinary calculus, and since then has been frequently found as the only or chief constituent of many calculi. UNGER and PRINSON have extracted it from guano, while SALOMON has shown it to be one of the products formed in the pancreatic digestion of fibrin, and also to be present in malt-sprouts. The latter has been recently confirmed by BALKE. SCHÜTZENBERGER found it together with carmin and hypoxanthin in the liquors from yeast. It is a normal constituent of the urine,

but is present only in extremely minute quantities. From 10,000 liters of urine KRÜGER and SALOMON isolated 13 g. of xanthin, 12.5 g. of paraxanthin, and 7.5 g. of heteroxanthin. During the use of sulphur-baths, or after the thorough application of sulphur salves, the quantity of xanthin in the urine is considerably increased. It is likewise more abundant in the urine of leucocythæmic patients, for the reasons already given on page 417. From small amounts of leukæmic urine (two cases) SALOMON, however, was not able to isolate it with the sodium-reaction. It was found in one out of four pneumonic urines; and in two out of ten normal urines from which at times it may be wholly absent. BAGINSKY holds that the amount of xanthin normally present in the urine may be increased tenfold in the case of acute nephritis. BENGE JONES observed in the urine of a child sick with renal colic a deposit of crystals which he considered to be xanthin, but other observers are inclined to regard the crystals as those of hypoxanthin. VAUGHAN has reported the presence of xanthin in deposits from the urine of patients with enlarged spleen. According to SCHINDLER, in the thymus gland and in the spermatozoa of the carp there are but very small amounts, if any, of xanthin, whereas the sarkin bases, especially adenin, are present in abundance. This observation has been confirmed by INOKO. The xanthin bases (xanthin and guanin) are present in variable but greater amount than the sarkin bases (adenin and hypoxanthin) in spermatozoa of bull, boar, salmon; in pancreas and nucleinic acid from bull's testicles. The bases rich in oxygen, as hypoxanthin and xanthin, are more abundant (2 to 1) than those rich in nitrogen (adenin and guanin) (INOKO). DRECHSEL isolated xanthin and cystin from the liver of the horse.

Xanthin can be obtained most conveniently by the action of nitrous acid on guanin. It was held by STRECKER that it may be obtained by reduction of uric acid with sodium amalgam according to the equation :



This view, however, has not been confirmed by FISCHER (see page 482). Recently (1895) FISCHER effected the synthesis of caffèin (trimethyl-xanthin) from dimethyl uric acid; also, the conversion of theobromin into the same uric acid derivative. The reverse operation, the conversion of hypoxanthin into xanthin, likewise reported by STRECKER, has not been confirmed by FISCHER or by KOSSEL. It is, therefore, evident that while these bodies apparently form a continuous oxidation series with uric acid as the final product, and although this change probably goes on in the animal economy, yet all attempts to reproduce it in the laboratory by oxidation with potassium permanganate or nitric acid have yielded only negative results. Again, xanthin may be prepared from guanin by putrefaction of the latter, or, as stated above, by oxidation with nitrous acid. The change may be represented by this equation :



This reaction, first described by STRECKER (1858), and later by FISCHER, corresponds exactly to the one by which KOSSEL has transformed adenin into hypoxanthin (see page 426).

GAUTIER, starting out on the hypothesis that xanthin is a polymerization-product of hydrocyanic acid, has endeavored to prepare it directly from this compound. Indeed, he claims to have succeeded in effecting the synthesis of not only xanthin, but also its homologue, by simply heating hydrocyanic acid in a sealed tube with water and a little acetic acid, the latter being added to neutralize any ammonia that might form. He expresses the reaction as follows :



Nearly all the methods that have been employed for the preparation of xanthin are based upon its precipitation as the insoluble silver compound. From the urine it can be isolated according to the method given under paraxanthin. It may also be obtained from the urine by Hofmeister's

method. The urine, acidulated with hydrochloric acid, is precipitated with phosphotungstic acid; the precipitate is decomposed by warming with baryta, filtered, and the filtrate is freed from barium by the cautious addition of sulphuric acid. The solution is then made alkaline with ammonium hydrate, any traces of phosphates that appear are filtered off, and finally it is precipitated by the addition of ammoniacal silver nitrate. The precipitate which forms consists of the silver compounds of the xanthin bodies, and is purified by dissolving in hot nitric acid, as given on page 419. Xanthin has been shown to be formed at the same time with guanin, adenin, and hypoxanthin, in the decomposition of certain nucleins or their nucleinic acids by means of dilute acids. It may, therefore, be prepared from cellular organs according to the method given under Adenin. For the isolation and estimation of xanthin, see page 480.

Xanthin is a white, granular, amorphous body, and is deposited from hot aqueous solution on cooling in colorless floccules, or as a fine powder, which, under the microscope, is seen to consist of rounded granules. When occurring in calculi, it forms compact, moderately hard, yellow, or brown fragments, which, on being rubbed with the finger-nail, assume a wax-like appearance; when isolated from the urine it is yellowish, and even when prepared from guanin it is still colored. The decoloration can be effected, according to BALKE, by converting the xanthin into the mono-sodium compound, which can be recrystallized and then decomposed with acetic acid. The base is thus obtained in snow-white floccules. It is difficultly soluble in cold water (about 14,000 parts), alcohol, and ether; somewhat more soluble in boiling water (about 1200 parts). It is soluble in alkalis and alkali carbonates, not bicarbonates, and from these solutions it is precipitated on neutralization with acids, or by passing carbonic acid. In warm ammonia it dissolves more readily than does uric acid or guanin, and on cooling the ammonium compound recrystallizes. The solubility in cold, even strongly acid solutions, is very slight (WULF). It acts

as a weak base and as a weak acid; with salts of the heavy metals it forms difficultly soluble or insoluble compounds. Its basic properties, however, are weaker than those of hypoxanthin or guanin.

When xanthin is evaporated with nitric acid it leaves a lemon-yellow residue (hence its name), which is not changed by ammonium hydrate—distinction from uric acid—but with potassium hydrate becomes yellowish-red, on heating purple-red. This, the so-called xanthin-reaction, is not given by hypoxanthin or by adenin. When added to a mixture of bleaching-powder and sodium hydrate in a watch-glass the solution becomes covered by a dark-green scum, which changes to a brown, and soon disappears—distinction from hypoxanthin.

By means of a very interesting synthetic reaction, xanthin may be converted into theobromin, the active constituent of *Theobroma cacao*. Thus, the xanthin is dissolved in a sufficient quantity of sodium hydrate, necessary to form the neutral compound $C_5H_2Na_2N_4O_2$, and this product, when treated with boiling acetate of lead, yields a white precipitate of lead-xanthin, $C_5H_2PbN_4O_2$. This is dried at 130° , then heated for twelve hours at 100° with methyl iodid, when the dimethyl-derivative, $C_5H_2(CH_3)_2N_4O_2$, is formed. This compound is identical with the natural theobromin, and by a similar treatment is converted into trimethyl xanthin or caffeine. The relation of xanthin to thein (caffein) is shown in the fact that it exists together with hypoxanthin, adenin, and possibly guanin, in fresh tea-leaves. It is, therefore, clear, that by starting from guanin or guano we can produce successively xanthin, methyl-xanthin, dimethyl-xanthin, and trimethyl-xanthin, the last two compounds being identical with the alkaloids of theobroma and of coffee.

On heating xanthin a small portion volatilizes; the greater part decomposes into ammonium carbonate, cyanogen, and hydrocyanic acid. Heated to 200° with hydrochloric acid, it decomposes with the formation of ammonia, carbonic acid, formic acid, and glycooll (E. SCHMIDT, see page 486). When

bromin is allowed to act on xanthin, there is formed a substitution-compound, having the formula $C_5H_3BrN_4O_2$. It may also be obtained by the action of nitrous acid on brom-guanin (page 467). With potassium chlorate and hydrochloric acid it yields alloxan and urea (page 485).

Xanthin is a weak base, which dissolves in acids with the formation of salts.

The hydrochlorid, $C_5H_4N_4O_2.HCl$, is difficultly soluble in water, more so than the corresponding salt of hypoxanthin, from which it is deposited in glistening six-sided plates, often forming aggregations. Its solution does not precipitate platinum chlorid. The nitrate forms fine yellow crystals.

The sulphate, $C_5H_4N_4O_2.H_2SO_4 + H_2O$, crystallizes in microscopic glistening rhombic plates, decomposable by water.

With baryta-water xanthin forms the difficultly soluble compound $C_5H_4N_4O_2.Ba(OH)_2$, which corresponds to the hypoxanthin salt $C_5H_4N_4O_2.Ba(OH)_2$, and to that of guanin.

On the addition of a very small amount of sodium hydrate to xanthin it dissolves, and very soon small white needles separate. The crystals dissolve in excess of alkali. This xanthin-sodium compound, $C_5H_3NaN_4O_2 + H_2O$, is also obtained by passing carbonic acid into an alkaline solution of xanthin. It forms small bunched needles, which are rather easily soluble in water, imparting an alkaline reaction. On the addition of acetic acid the pure white base is thrown down. The compound is partly dissociated by hot water, and resembles the corresponding primary uric acid salts. It is probable that xanthin can form, like uric acid, a soluble secondary salt, since with excess of sodium hydrate it forms a readily soluble compound, which probably contains two atoms of sodium. For the reactions of this compound, see page 452. (See heteroxanthin and paraxanthin.) It does not give a mono-methyl xanthin by heating with methyl iodid (BALKE). The water of crystallization is expelled only at 190° – 200° .

From ammoniacal solution silver nitrate precipitates the compound $C_5H_4N_4O_2.Ag_2O$, which is insoluble in ammonia, but soluble in hot nitric acid. From the nitric acid solution, on

long standing, there separates the compound $C_5H_4N_4O_2 \cdot AgNO_3$, which, on contact with water, decomposes, giving off nitric acid. The ammoniacal solution is also precipitated by lead acetate—separation from hypoxanthin—also by calcium and zinc chlorids. Cupric acetate gives a precipitate only on boiling. The aqueous solution is not precipitated by lead acetate, but is by phosphomolybdic acid, phosphotungstic acid, by mercurous and mercuric salts. Picric acid gives an easily soluble compound, which resembles that of hypoxanthin, but differs from that of guanin. Xanthin gives with copper solutions and reducing substances (Drechsel's reaction, see page 425) a milk-white precipitate which eventually becomes bluish-green (BALKE). Since xanthin has more imido-groups than adenin or hypoxanthin and less than uric acid, it is probable that the solubility of the copper compound will be between the solubilities of the corresponding compounds of hypoxanthin and uric acid; that is, between 1 : 250,000 and 1 : 360,000, the solubilities respectively in hot water. Uric acid (copper compound) is soluble in 560,000 parts of cold water (KRÜGER).

As to the physiological relation of xanthin very little need be said. It bears the same relation to guanin that hypoxanthin does to adenin, and, like the latter, is to be looked upon as an intermediate compound, a step lower than guanin, and nearer the limit of oxidation—uric acid. It is quite probable that in the body it is oxidized about as rapidly as it is formed. Like hypoxanthin, it is to be regarded as a true muscle stimulant, especially of the heart (BAGINSKY). According to FILEHNE, it produces in frogs a decided muscular rigor and paralysis of the spinal cord. The heart muscle is also affected, which is not the case with caffein or theobromin. The fatal dose is less than one-half pro mille. In its action it is stronger than theobromin, while caffein is weaker than either of the two. PASCHKIS and PAL hold that the reverse is true.

Isolation and estimation of the xanthin bases.

Neubauer's method, which has been employed in some modification or other for the isolation of xanthin bases, de-

depends upon the fact that all xanthin bases are precipitated from an ammoniacal solution by an ammoniacal silver solution. This reaction, as KRÜGER has pointed out, is given by all xanthin bases which contain an imido-group capable of substitution. It is not given by caffein, the fully methylated xanthin, or by dimethyl-hypoxanthin. The further separation of the bases is accomplished by dissolving the silver precipitate, together with a little urea, to destroy any nitrous acid, in boiling nitric acid of 1.1 specific gravity. The hot solution is filtered, and from the filtrate on cooling the "hypoxanthin fraction" of SALOMON, hypoxanthin, guanin, carnin, adenin, and episarkin, crystallize as the corresponding silver nitrate compounds. The portion of silver salts remaining in solution in the nitric acid, the "xanthin fraction," consists of xanthin, paraxanthin, and heteroxanthin.

The application of this method to urine, and especially the separation of the latter three bases, is fully described on page 471. The method of extraction from meat, glands, etc., is given on page 419.

The separation of the bases of the hypoxanthin fraction is given on pages 419-422.

The usual method, then, for the separation of the four common xanthin bases is to dissolve the silver precipitate obtained from ammoniacal solution in boiling nitric acid of 1.1 sp. g. The filtrate, on cooling, yields a precipitate of the silver nitrate compounds of adenin, hypoxanthin, and guanin. The xanthin compound remains in solution. The silver salts of the three bases mentioned are decomposed with dilute hydrochloric, or with hydrogen or sodium or ammonium sulphide, and the acid solution of the three bases is then heated on the water-bath with excess of ammonia. Guanin is thus thrown out of solution (page 419). In the ammoniacal filtrate the adenin is separated from the hypoxanthin and estimated by picric acid (see page 422). The hypoxanthin is estimated as hypoxanthin silver picrate (page 448). Instead of the separation of guanin from the three bases by ammonia, WULF's metamorphosphate method may

be used (page 462). The adenin and hypoxanthin are then precipitated from the filtrate by ammoniacal silver solution. The silver salts decomposed with hydrochloric acid, and in the filtrate the adenin can be estimated as the picrate; the hypoxanthin as the hypoxanthin silver picrate.

Again, guanin may be estimated according to the copper method of BALKE.

Adenin and hypoxanthin are separated by precipitation in the cold with copper sulphate and sodium hyposulphite (KRÜGER).

A second method for the extraction of the xanthin bases is based upon DRECHSEL's reaction, namely, that they are precipitated by copper solutions in the presence of reducing agents (see page 425). The precipitation is so complete that the filtrate does not react with ammoniacal silver nitrate. The reliability of the process has been quantitatively tested by BALKE, who found the yield of xanthin bases from meat-extract to be slightly greater by the copper method over NEUBAUER's silver nitrate process. In addition to cheapness the method possesses an advantage in the less bulky precipitates. KRÜGER has also applied this method advantageously for the isolation of adenin from tea-extracts.

All the xanthin compounds, containing an imido-group capable of substitution, with the exception of theobromin, are precipitated. Caffein and dimethyl-hypoxanthin contain no imido group, and are therefore not precipitated, creatin and creatinin are not thrown down. The more imido-groups present in a compound the less soluble is the precipitate.

The method as applied by BALKE to a fresh meat-extract was as follows: The clear solution is rendered alkaline with sodium hydrate and the slight precipitate of phosphates removed by filtration. To the filtrate, hydroxylamin hydrochlorid is added, and then gradually Fehling's solution. The flocculent yellowish-brown precipitate is washed by decantation with a solution of sodium acetate, and then filtered. The precipitate is suspended in water, to which some ammonia is added, and decomposed with hydrogen sulphid.

The filtrate from the copper sulphid is concentrated, rendered ammoniacal, and precipitated with basic lead acetate. Lead compounds of carmin, hypoxanthin, and xanthin are precipitated completely, since the filtrate from the lead precipitate gives no reaction with silver nitrate. The precipitate is repeatedly boiled with water to remove the soluble carmin-lead compound. This solution is decomposed with hydrogen sulphid, and proceeded with according to Weidel's method for carmin.

The insoluble lead salts are decomposed with hydrogen sulphid; the filtrate on concentration yields a yellowish-white mass of xanthin and hypoxanthin. These can now be separated by conversion into the silver salts and solution in nitric acid of 1.1 specific gravity.

The copper method is of special value in the examination of vegetable extracts for xanthin bases. Owing to the abundant presence of reducing substances the silver method is scarcely applicable. BALKE examined malt-sprouts by this method. The sprouts were boiled for several hours with 0.5 per cent. H_2SO_4 , filtered, concentrated to a small bulk, and the dark solution precipitated with neutral lead acetate, to remove sulphates, humus-substances, etc. The filtrate is decomposed with hydrogen sulphid, again heated to about 70° – 80° , and copper sulphate solution gradually added with stirring. The addition of a reducing substance is unnecessary. The flocculent precipitate is allowed to settle, then filtered. To purify the copper precipitate it is then suspended in water, decomposed with hydrogen sulphid, filtered, and the filtrate again precipitated, as above, with copper sulphate. The purified copper precipitate is again decomposed, the filtrate rendered ammoniacal, and precipitated with silver nitrate. The flocculent silver precipitate is then treated with boiling nitric acid of 1.1 specific gravity in the usual way.

The copper method may be employed for the volumetric estimation of the xanthin bodies. Thus, ARTHAUD and BUTTE have devised a method for the titration of uric acid as cuprous urate. BALKE applied it to the estimation of

guanin. To the alkaline solution of guanin a solution of hydroxylamin hydrochlorid is added, and Fehling's solution gradually added from a burette. The end-reaction is attained when the yellowish-white precipitate is turned yellowish red by the cuprous oxid derived from the excess of copper. The precipitate is assumed to be the pure cuprous oxid compound, $C_5H_5N_3O.Cu_2O$. The results are a trifle low. The difference with 1 per cent. solution is about 1 per cent. With very dilute solutions the error is much greater.

KRÜGER obtained good results by precipitating adenin, as well as hypoxanthin, with copper sulphate and sodium bisulphite, collecting the precipitate on a weighed filter, and drying at 100° to constant weight. From the nitrogen as determined by Kjeldahl's method the amount of adenin is calculated. Excellent results are also obtained with uric acid, which, however, is not precipitated by copper sulphate and sodium hyposulphite. The cuprous, as well as the silver compound of uric acid and the xanthin bases, are soluble in excess of sodium hyposulphite.

Recently (1894) KRÜGER and WULF have perfected a copper method for the estimation of the alloxuric bodies and bases (see page 482) in the urine. The method can be applied to extracts of organs as well. Copper sulphate and sodium hyposulphite is a specific reagent for the alloxuric bodies, since urea, creatin, creatinin, amido-acids, pepton, albumose, allantoin are not precipitated. The amount of nitrogen in the alloxuric bases is found by subtracting the amount of nitrogen in the uric acid found by the Salkowski-Ludwig method from the amount of nitrogen in the alloxuric bodies precipitated by the copper method. The reagents employed are a 13 per cent. solution of copper sulphate and a solution of sodium bisulphite (1-2).

100 c.c. of the urine, freed from albumin, is placed in a beaker and boiled; then 10 c.c. of sodium bisulphite solution and 10 c.c. of the copper solution are added and the whole raised to boiling. Finally, 5 c.c. of a 10 per cent. solution of barium chlorid are added. This causes the precipitate to

settle rapidly and permits washing. The precipitate is allowed to stand two hours, then filtered through a 10–12 cm. Swedish plaited filter and washed about five times with warm water (60°). The filter and its moist contents are placed in a Kjeldahl round digestion-flask (150 c.c.), and 15 c.c. of concentrated sulphuric acid added, together with 10 g. of potassium sulphate and 0.5 g. copper sulphate. On boiling for about one hour the solution becomes clear. The solution is then transferred to a flask, rendered alkaline with sodium hydrate, and distilled. Talc can be advantageously used to prevent bumping. The distillate is titrated with $\frac{N}{10}$ oxalic acid, using rosolic acid as an indicator.

By subtracting now from the nitrogen thus obtained, the nitrogen calculated from the uric acid estimated by the Sal-kowski-Indwig method, the difference gives the nitrogen in the alloxuric bases in 100 c.c. of the urine. According to BAGINSKY, the 2.8–3.8 mg. of xanthin bases are present in 100 c.c. of urine. This corresponds to 0.042–0.057 g. per day. KRÜGER and WULF found by the method just given that on an average 0.1325 g. of alloxuric bases was excreted in the urine per day. The proportion of uric acid nitrogen to the nitrogen of the alloxuric bases was, on an average, 3.82 : 1.

In a case of leukaemia, BONDZYNSKI and GOTTLIEB found this proportion to vary from 1.06 : 1 to 3.22 : 1. The daily excretion of alloxuric bases was 0.5–0.6 g.

Separation of uric acid from xanthin bases.

In the precipitation of uric acid by the silver method the xanthin bases if present are likewise thrown down. However, as adenin and hypoxanthin are readily soluble in acids and alkalis, they are readily separated after decomposition of the silver precipitate. Xanthin and guanin are less soluble, and hence may render the uric acid impure, especially when this is isolated from animal organs. WULF detects the presence of xanthin in a uric acid precipitate by destroying the uric acid with hot dilute nitric acid—the xanthin remaining unchanged. The substance is heated on the water-bath with

dilute nitric acid (100 parts water and 5 parts nitric acid, 1.14 sp. g.), and after gas ceases to be given off it is boiled for a short time, rendered ammoniacal, and if xanthin is present it gives a precipitate with silver nitrate. The precipitate can be collected on a weighed filter, dried at 120° , and weighed as $C_5H_4N_4O_2 \cdot Ag_2O$; or from the weight of silver after ignition the amount of xanthin calculated. If the amount of xanthin is large, it can be weighed directly on weighed filter and dried at 110° by rendering the solution slightly ammoniacal after the oxidation with nitric acid, then acidulating with acetic acid and adding equal volume of alcohol. After twelve hours filter, wash with dilute alcohol, dry at 110° , and weigh.

HORBACZEWSKI'S method for the separation of uric acid from xanthin is as follows: The mixture of the two substances is dissolved with aid of gentle heat in a platinum dish in concentrated sulphuric acid (2 c.c. for 0.1 g. substance). The solution is diluted with four parts of water, stirred thoroughly, and set aside for 3–6 hours. The uric acid is thus precipitated, and is then collected on a small filter, washed with water, acidulated with sulphuric acid, then with pure water. Then it is transferred to the dish in which it was precipitated, dissolved in a little pure sodium hydrate (caustic), strongly acidulated with hydrochloric acid, and evaporated to a few c.c. After standing one hour it is filtered through Ludwig's glass-wool filter, washed with HCl-water, then with water, finally with alcohol and ether, dried at 110° , and weighed. The filtrate and wash-water are combined, and a correction is made for the solubility (1 : 16,000) of uric acid. This correction is added to the weighed amount. The results obtained by this method are excellent. The separation from guanin and xanthin is complete.

Constitution of the bases of the uric acid group. These bases are commonly spoken of as xanthin bases or as nuclein bases, since they are derived from the nucleins. The nucleins vary chemically among themselves, but in general they are decomposable into an albumin and into a nucleinic acid. The

latter on decomposition with acids, as a rule, yields one or more of the bases of the uric acid group. Although considerable similarity exists between the four members of the group, it does not follow that any one of the bases can be converted successively into all of the others. As shown in the preceding pages, adenin is readily changed into hypoxanthin and guanin into xanthin. The statement made by STRECKER that hypoxanthin by the action of fuming nitric acid yields a nitro-product, which on reduction gives xanthin, was supposed to show that hypoxanthin possessed a constitution similar to that of xanthin. KOSSEL, however, showed in 1882 that this conversion of hypoxanthin into xanthin did not take place, and in this he was confirmed by FISCHER in 1884. In the same way the observation of RHEINECK, reported by STRECKER, that uric acid is converted by sodium amalgam into xanthin, and this still further reduced to hypoxanthin, has been shown to be incorrect by FISCHER, who failed to change xanthin into uric acid or uric acid into xanthin. While, therefore, uric acid, xanthin, and hypoxanthin contain three, two, and one atoms of oxygen, respectively, and are structurally closely allied, nevertheless thus far it has not been possible to convert any one of those compounds into the others. For this reason KOSSEL suggested that the nuclein bases be divided into two groups: (1) *Xanthin* bases, which include guanin, xanthin, and its methyl-derivatives; (2) *Sarkin* bases, which include adenin, hypoxanthin, and their methyl-derivatives. Uric acid may be said to constitute a third group, standing as it does by itself, also derived undoubtedly from nuclein.

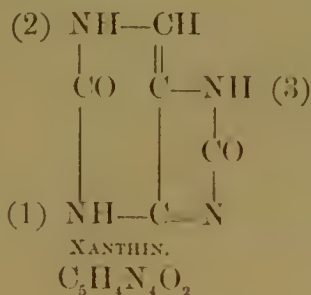
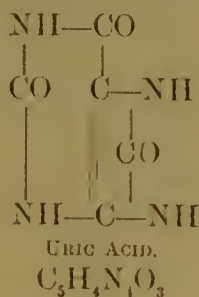
More recently (1894) KOSSEL and KRÜGER introduced a new general term based upon the fact that uric acid and the xanthin bases contain alloxan and urea-residues. Alloxuric bodies, therefore, include uric acid and the xanthin bases. On the other hand, alloxuric bases include xanthin, guanin, adenin, hypoxanthin, heteroxanthin, paraxanthin, theobromin, theophyllin, caffèin, earnin. Episarkin would not be included in this group, as it probably has only an alloxan residue.

The classical investigations of FISCHER have shown that there

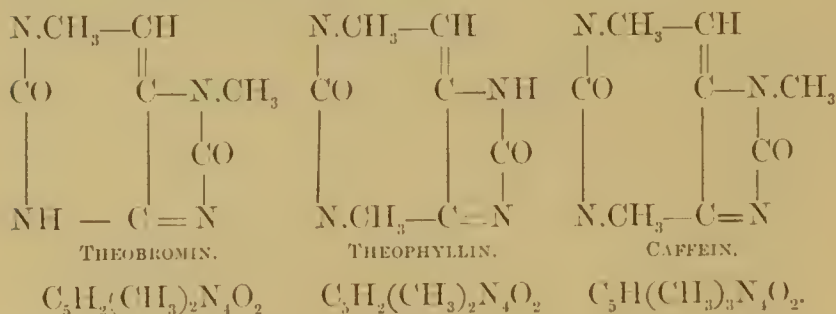
is a close structural relationship between uric acid and the xanthin bases, as mentioned above. On the other hand, by the failure to convert hypoxanthin into xanthin, or the reverse, the constitution of hypoxanthin and of adenin has remained uncertain. The recent investigations of KRÜGER, however, have furnished the experimental proof of the relationship of the sarkin bases to the xanthin bases and to uric acid.

The conversion of a member of the xanthin group into a uric acid derivative and the reverse change have at last been accomplished by FISCHER (*Berichte*, **28**, 2473, 2480, 3135). Brom-theobromin was changed to δ -dimethyl uric acid. The latter compound on treatment with phosphorus pentachlorid yields a purin body identical with that obtained from theobromin and caffenin by similar treatment. γ -dimethyl uric acid with this reagent, however, gave chlor-theophyllin, which on reduction with hydriodic acid gave theophyllin. By introducing a methyl group into the latter caffenin was obtained. The complete synthesis of theophyllin and caffenin has, therefore, been accomplished.

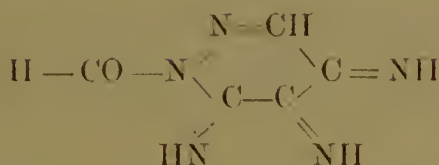
In 1881 FISCHER showed that caffenin possessed the structural formula as given below. In the following year he succeeded in changing xanthin by heating xanthin-lead with methyl iodid into theobromin, and thus, once for all, established the constitution of xanthin, since the silver compound of theobromin, as shown by STRECKER, on treatment with methyl iodid, yields caffenin. These investigations therefore demonstrated that theobromin is a dimethyl-xanthin, and that caffenin is a trimethyl-xanthin. In his second paper on uric acid in 1884 FISCHER was able definitely to establish the constitution of uric acid.



By replacing a hydrogen atom in either one of the three imido-groups, 1, 2, or 3, in xanthin by a methyl-group a mono-methyl-xanthin results. Three such derivatives are possible, and it is probable that heteroxanthin, $C_6H_6N_4O_2$, is one of these. This has, indeed, been demonstrated by KRÜGER and SALOMON (1895), who showed that it is xanthin with a methyl-group in position 3. By the introduction of two methyl-groups into the xanthin molecule three compounds result, according as the substitution is 1 and 2, 1 and 3, or 2 and 3. Theobromin results from substitutions in the imido-groups, 2 and 3. Theophyllin, a new base isolated by KOSSEL in 1888 from the caffeine mother-liquors from tea-leaves, and synthesized, as stated above, by FISCHER in 1895, is also a dimethyl-xanthin, and its silver salt heated with methyl iodid yields caffeine. On oxidation it yields dimethyl-alloxan, and hence the methyl groups occupy positions in the imido-groups, 1 and 2. Paraxanthin, $C_7H_8N_4O_2$, is probably a third dimethyl-xanthin, but its constitution has not been established. Because of its relation to heteroxanthin it may be an ethyl-xanthin. By substituting three methyl-groups in xanthin in the imido-groups, 1, 2, and 3, caffeine results.

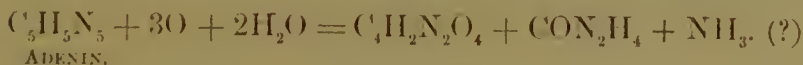
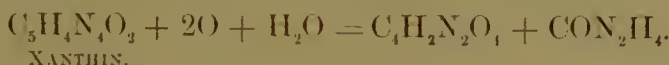


GAUTIER, starting out with the idea that they are polymerization-products of hydrocyanic acid, has deduced theoretically cyclic formulæ, recalling the hexagon of the benzol derivatives. These formulæ, though formidable in appearance, are a complete failure so far as they are expressions of chemical reactions. Thus, the formula of guanin :



fails to show either a urea or a guanidin-residue, and yet it is a well-known fact that guanin on oxidation yields parabanic acid and guanidin (page 464). In a similar manner, his xanthin formula fails to show up the urea-residues which we know to be present.

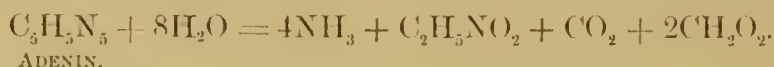
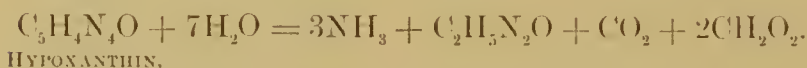
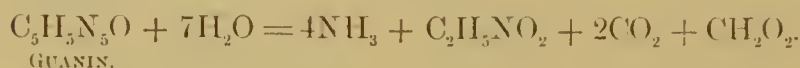
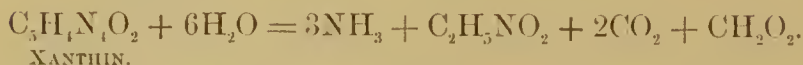
In 1891 and 1893 KRÜGER established a chain of evidence which connects adenin and hypoxanthin with uric acid and xanthin in an indisputable manner. Thus, the bromine-derivatives of adenin and of hypoxanthin correspond to those of xanthin, guanin, and caffen; on oxidation with chlorine yields alloxan and urea. The analogy to the other members of the uric acid group is seen from the following equations:



The equations for adenin and hypoxanthin are not fully established, but the bromine compounds are decomposed into alloxan. In addition to this urea and oxalic acid are formed, both of which may be derived from alloxan by decomposition, though some of the urea may be independent of the breaking down of the alloxan group.

Again, adenin and hypoxanthin on decomposition with concentrated hydrochloric acid yield the same products (glycocoll, ammonia, formic and carbonic acids) as xanthin,

guanin, etc. This decomposition is best seen from the following equations:



Uric acid has no CH group, hence does not yield formic acid, whereas xanthin and guanin have each one CH group and yield one molecule of formic acid. Hypoxanthin and adenin therefore have two CH groups.

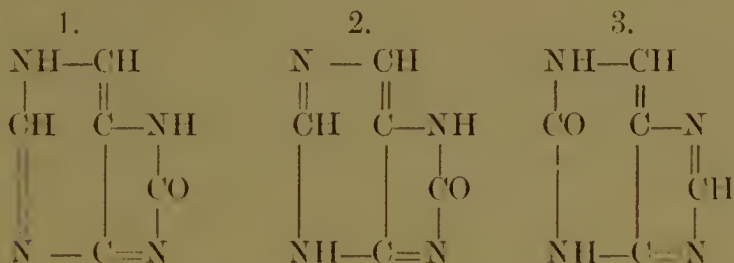
Furthermore, uric acid contains three CO groups and yields three CO₂ molecules, whereas xanthin and guanin have two CO groups and yield two CO₂ molecules. Hence, adenin and hypoxanthin have but one CO group, since they yield but one CO₂ molecule.

The existence of dimethyl-hypoxanthin shows that hypoxanthin has two imido-groups capable of substitution by alkyls. Adenin therefore has three NH groups, one of which is replaced by oxygen when the base is changed to hypoxanthin. Again, uric acid has three atoms of O and four replaceable NH groups, while xanthin has two atoms of O and three replaceable NH groups. Hypoxanthin therefore with one atom of O should have two NH groups. This analogy does not extend to adenin and guanin. Hypoxanthin consequently contains two CH, one CO, two NH, and an alloxan group.

The bromine-derivatives of hypoxanthin, adenin, xanthin, guanin, and caffein result by the substitution of bromine in the CH group of xanthin.

As pointed out by FISCHER, hypoxanthin possesses one of

three formulæ, depending on which of the two CO groups in xanthin is replaced by the CH group. The three possible formulæ of hypoxanthin are:



To decide positively as to which of these three formulæ belongs to hypoxanthin is as yet impossible. KRÜGER considers the third formula as the least probable for the reason (1) that brom-hypoxanthin yields but a very small amount of alloxan, whereas according to formula 3 it should yield as much as xanthin; (2) that the dimethyl-hypoxanthin is decomposed by hydrochloric acid, yielding methylamin and methyl-glycocoll, according to the equation given on page 455. KRÜGER, however, assumes, with FISCHER, that gly-

cocoll is derived from the atom complex $\begin{array}{c} \text{C}-\text{NH} \\ | \\ \text{C} \end{array}$. Con-

sequently the dimethyl compound, according to formula 3, should yield two molecules of methylamin and one of glycocoll. There is no evidence, however, to exclude the group

$\begin{array}{c} \text{C} \\ | \\ \text{NH}-\text{C} \end{array}$ from consideration as the glycocoll residue.

Formula 1 can yield methyl-glycocoll only from the group

$\begin{array}{c} \text{C}-\text{NH} \\ | \\ \text{C} \end{array}$, but formula 2 can yield it either from this group

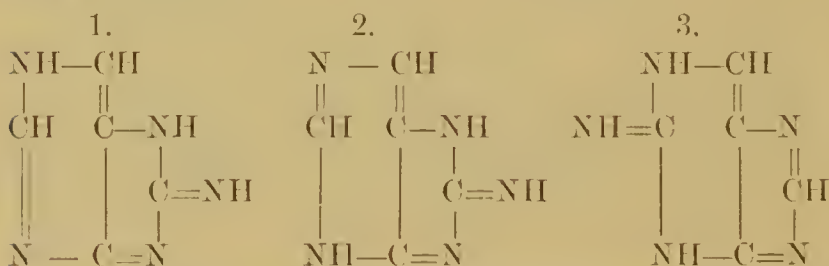
or from the group $\begin{array}{c} \text{C} \\ | \\ \text{NH}-\text{C} \end{array}$, and, lastly, the formula 3 can

yield it from the last group. The fact that brom-hypoxanthin yields on oxidation but a small amount of alloxan, cannot decide against formula 3 until it has been shown that brom-

xanthin on oxidation yields a much larger amount. The oxidation of brom-hypoxanthin cannot be compared direct with that of xanthin.

Apparently the only means of deciding between these formulæ is to oxidize the dimethyl-hypoxanthin. Formulæ 1 and 2 should yield mono-methyl alloxan and mono-methyl-urea, whereas formula 3 should yield dimethyl-alloxan and urea. KRÜGER employs the first formula to designate hypoxanthin. The methyl groups in the dimethyl compound therefore are introduced into the imido-groups 2 and 3 as indicated on page 483.

The constitution of adenin will depend upon which one of the three possible formulæ of hypoxanthin is the correct one. For the present, then, we have three possible structural formulæ of adenin :

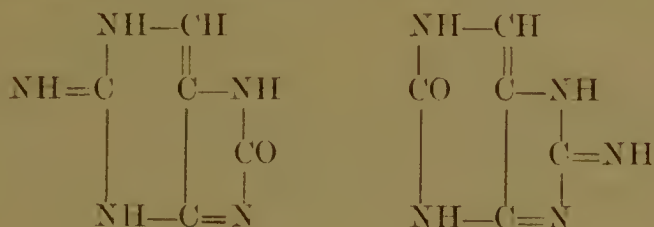


Formula 1 is the one assumed by KRÜGER. Methyl-adenin on decomposition with hydrochloric acid yields methylamin and glycocoll (see page 440). The methyl group is therefore placed in imido-group 2 (see xanthin formula). It would occupy the same position in formula 3, which will also allow

the formation of glycocoll from the group $\begin{array}{c} \text{C} \\ | \\ \text{NH}-\text{C} \end{array}$. For-

mula 3, compared with the commonly accepted one of guanin (1), should on oxidation yield nearly the same products as guanin on oxidation, namely, guanidin, carbonic acid, and possibly parabanic acid. In one oxidation experiment KRÜGER obtained from 17 g. of brom-adenin only about 1 g. of alloxan. From a second experiment with 20.6 g. brom-adenin no alloxan was obtained.

Constitution of guanin. The fact that guanin is readily changed into xanthin shows that the two bases are closely related. In fact, the relation is the same as that between adenin and hypoxanthin. By decomposition with concentrated hydrochloric acid WULF obtained the same products as are given by xanthin. On oxidation, however, with chlorine neither STRECKER nor FISCHER were able to obtain alloxan and urea, but instead obtained parabanic acid, guanidin, and carbonic acid. A guanidin-residue is contained in the molecule in place of an urea-residue in xanthin. Two formule are therefore possible for guanin ;



On account of the behavior to chlorine FISCHER considered the first formula as the more probable. It will be observed, furthermore, that the second formula should yield alloxan the same as xanthin. The difficulty of preparing alkyl-derivatives of guanin, compared with the ease with which the other xanthin compounds form them, renders it as yet impossible to decide between the two formule. FISCHER and REESE failed in preparing alkyl-derivatives, and WULF succeeded only with ethyl-guanin.

HETEROXANTHIN, $\text{C}_6\text{H}_6\text{N}_4\text{O}_2$, is a new base which was isolated from the urine in 1884 by SALOMON, and again in 1893 by BALKE. In its composition it is methyl-xanthin, and is intermediate between xanthin and paraxanthin or dimethyl-xanthin. It occurs in the urine of man and of the dog in about the same amount as paraxanthin, and the method for its isolation will be found under the description of that base. It is a remarkable fact that this base occurs in dog's urine unaccompanied by paraxanthin, and the same seems to hold

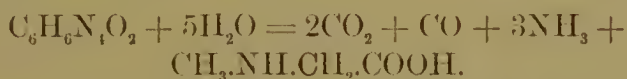
true for the urine of leucocythæmic persons. SALOMON examined the liver, muscles, and kidneys of a dog, but was unable to obtain any heteroxanthin or paraxanthin, and the total amount of xanthin bodies present was about normal. Hence, he is inclined to think that these two bases may possibly have their origin in the kidney. Paraxanthin and heteroxanthin are not present in the urine or kidneys of the cow. SALOMON found this base in the urine of one out of two leucæmic patients; in one with splenic tumor, and three times out of ten normal urines. Unlike the other xanthin bodies, heteroxanthin has not as yet been isolated from plants, meat-extract, or guano. The amount of xanthin bodies present in the urine is unaffected by phosphorus-poisoning. Neither this base nor paraxanthin has been found in bull's testicles; xanthin is also absent, and only hypoxanthin and guanin were found to be present. ISOKO found in the semen of bull the four bases, adenin, guanin, xanthin, and hypoxanthin. In the nucleinic acid from bull's testicles 6.039 per cent. of xanthin was found; guanin was absent. The amount of hypoxanthin was also large, 1.96 per cent. against 0.736 per cent. of adenin.

From 10,000 liters of urine KRÜGER and SALOMON (1895) obtained 13 g. of xanthin, 12.5 g. of paraxanthin, and 7.5 g. of heteroxanthin.

Heteroxanthin forms a white amorphous powder, which sometimes on prolonged contact with water forms microscopic crystalline tufts. BALKE also described a crystalline modification. It is very difficultly soluble in cold water; much more easily in hot water, and the solution thus obtained is neutral in reaction. It is easily soluble in ammonium hydrate, but is insoluble in alcohol and ether. Like uric acid, it is readily soluble in piperazin. When heated it volatilizes without melting, and at the same time gives off a small quantity of hydrocyanic acid. On evaporation with nitric acid on the water bath (xanthin-reaction) it remains as a pure white residue, which on contact with sodium hydrate develops only a trace of reddish coloration or none at all. Weidel's

test (page 424) produces a splendid red color, which becomes blue on the addition of sodium hydrate. Simple evaporation with chlorine-water gives a similar though not so strong a color-reaction.

On heating with concentrated hydrochloric acid, or with dilute sulphuric acid, at 180°–200° it decomposes and yields sarkosin and other products, according to the equation (KRÜGER and SALOMON):



On treatment with methyl iodid it yields caffenin. For its structure, see page 484.

Silver nitrate produces in ammoniacal as well as in nitric acid solutions a precipitate which readily dissolves on warming in even very dilute nitric acid; from this solution, if not too concentrated, the heteroxanthin silver nitrate compound crystallizes in well-formed plate-like prismatic crystals. Copper acetate produces in the cold, in solutions of heteroxanthin, a clear-green precipitate. It is also precipitated by phosphotungstic acid, and by ammoniacal basic lead acetate. Pieric acid does not give a yellow-colored precipitate in solutions of the hydrochlorid. Mercuric chlorid readily precipitates heteroxanthin in the form of a grayish-yellow compound, which standing twelve or twenty-four hours becomes converted into pure white crystalline aggregations. This mercuric compound can be converted directly into the corresponding silver compound by the addition of silver nitrate and ammonia, as described under paraxanthin. For Drechsel's reaction with copper, see page 425. The precipitate that forms is gelatinous, milk-white, but soon turns green, as in the case of guanin and xanthin. The solubility of the copper compound would be about the same as of adenin and hypoxanthin, since it has two imido-groups.

The hydrochlorid is characterized by its rather difficult solubility and ready crystallization (a distinction from the paraxanthin salt). The salt forms large colorless tufts of

crystals, which on contact with water soon lose their transparency and become opaque ; gradually their crystalline form disappears, till finally they completely decompose with the formation of heteroxanthin. This decomposition is hastened by warming, either with or without addition of ammonia. Platinum chlorid produces in the hydrochloric acid solution a precipitate of crystalline double salt.

This base resembles paraxanthin in its property of yielding a difficultly soluble precipitate with the fixed alkali. This reaction is best brought about by dissolving the heteroxanthin hydrochlorid in warm dilute sodium hydrate, when, on cooling, the corresponding sodium salt will crystallize out in oblique-angled plates ; or clear long prisms. Long-pointed twin crystals are the most characteristic. The crystals show greater variations in form than those of paraxanthin. The behavior of the twin crystals with polarized light is of service in identification. These crystals dissolve easily in water, and on neutralization of the solution with an acid a dense pulverulent precipitate of heteroxanthin forms. The sodium compound, as well as that of paraxanthin, is permanent, non-deliquescent ; on moderate heating becomes cloudy, and melts above 300° . It is more difficultly soluble in water than the paraxanthin compound ; the solution has an alkaline reaction. It is soluble in mineral acids and ammonia, the latter redeposits it unchanged on evaporation. On neutralization of its solution with mineral acids, lactic, or acetic and carbonic acids, the pure base separates out as amorphous or crystalloidal roundish masses, while paraxanthin under the same conditions forms its characteristic crystals. The sodium is also removed from both compounds by borax, potassium bisulphate, biphosphate of sodium, bisulphite of sodium and potassium bicarbonate, ammonium bitartrate. Ammonium chlorid, nitrate, sulphate, carbonate, oxalate, tartrate, are transposed to form sodium salts, and the free bases are thrown down. Similar decomposition of the sodium salts of the two bases occurs when placed in urine or in meat-extract.

For illustrations of the sodium compounds of the two bases, see Virchow's *Archiv*, **125**, 556.

The potassium compounds of heteroxanthin and paraxanthin are well-crystallized bodies, of high melting-point, and are more soluble than the sodium compound. Their decompositions are the same as those of the sodium salt.

The reaction with sodium is the basis for SALOMON'S method of recognition of these bases in small quantities of urine. (See page 498.)

It can thus be distinguished from paraxanthin, the sodium compound of which, on similar treatment, yields the characteristic crystalline form of the free base. This sodium reaction, therefore, distinguishes it at once from xanthin, hypoxanthin, guanin, and paraxanthin. It differs from the latter, as has already been indicated, in the solubility and amorphous character of the free base; in the behavior of the hydrochlorid and the sodium compound, and in the not giving a precipitate with pieric acid, nor the characteristic odor given by paraxanthin on heating.

In its composition heteroxanthin is, as has already been stated, a methyl-xanthin, and may be related to if not identical with an isomeric body obtained synthetically by GAUTIER. The fact, nevertheless, remains that in the urine we have normally a homologous series of xanthin bodies, namely, xanthin, heteroxanthin, and paraxanthin. It is possible, as BONDZÝŃSKI and GOTTLIEB have pointed out that heteroxanthin results from the splitting up of more complex xanthin bodies introduced into the body with vegetable food. This origin is indicated in the minute amounts that it exists in the urine, in the fact that it has not been found in the decomposition of nuclein, and in the further fact that caffein and theobromin are excreted by the urine as methyl-xanthin.

The physiological action of heteroxanthin has been studied by KRÜGER and SALOMON (1895). Its action is almost the same as that of paraxanthin (page 502), indicating a close chemical relation. Its action, however, is much less intense.

A dose two to three times greater than that of paraxanthin must be injected into frogs to produce the same symptoms. It has a local action producing early contraction and rigor of the muscles. Its general action is seen in the gradual or rapid paralysis of respiration, according to the dose; in the loss of motion in the extremities, and in the decrease of reflexes.

METHYL-XANTHIN, $C_6H_6N_4O_2$. This compound, isomeric if not identical with heteroxanthin, was isolated in 1895 by BONDZÝNSKI and GOTTLIEB from the urine of dogs, rabbits, and men after administration of large doses of theobromin. Ingestion of large doses of caffein likewise seems to give rise to this base. 19 per cent. of the theobromin fed to a rabbit appeared in the urine as such; whereas 24.6 per cent. was excreted as methyl-xanthin. When caffein is fed the per cent. of methyl-xanthin is less.

It separates from hot-water solution on cooling in crusts, or in the form of microscopic prisms, or as long needles. On rapid concentration of its solution it is thrown down in an amorphous condition; likewise by addition of acetic acid to its solution in alkalis. The amorphous floccules soon change to crystals. From the solution in sodium hydrate it is precipitated by ammonium salts. It melts at near 310° with decomposition and sublimation. The solubility in water at 18° is 1:1592; at 100° is 1:109; in absolute alcohol at 17° , 1:7575; at 100° , 1:2250. It is insoluble in chloroform.

It is precipitated by copper sulphate and sodium hyposulphite as a flocculent greenish-white precipitate, which later becomes brownish. Silver nitrate produces in ammoniacal solution a gelatinous precipitate, insoluble in excess of ammonia, readily decomposed by warming with hydrochloric acid. From solutions in hot sodium hydrate it separates as the sodium salt, $C_6H_5NaN_4O_2 + 4H_2O$, which crystallizes in very long rhombic plates and prisms. The barium salt, $(C_6H_5N_4O_2)_2Ba$, is formed by dissolving the base in barium hydrate, or by addition of barium chlorid to the solution

in alkalis. When recrystallized it forms rosettes or balls of crystals.

On heating the silver compound with methyl iodid and methyl alcohol in a sealed tube at 100° it yields caffèin.

It gives Weidel's test, but not the xanthin-reaction.

PARAXANTHIN, $C_7H_8N_4O_2$, was isolated in 1883 by SALOMON, who has since shown it to be a constituent of normal urine, although present in exceedingly minute quantity. Thus from 1200 liters of urine only 1.2 grams (0.0001 per cent.) of this substance were obtained. It has not been found in the urine of dogs or in that of leucocythæmic patients. By the sodium-reaction SALOMON failed to detect paraxanthin in the urine of two leukaemic patients, one with splenic tumor, and four pneumonia cases. On the other hand, it was found in nine out of ten normal urines, twice accompanied by heteroxanthin. The proportion of the two bases varies: Thus from 5 liters of one normal urine 10 mg. of well-crystallized paraxanthin and as much heteroxanthin were obtained. THUDICHUM was the first to isolate paraxanthin from urine, and he named it urothecobromin (1879). It has been met with again by BALKE in 1893.

The method employed for the isolation of this base is, with a slight modification, that of E. SALKOWSKI, as originally proposed for the preparation of xanthin bases from urine. The urine in portions of 25 to 50 liters is made alkaline with ammonium hydrate and allowed to stand twenty-four hours. The clear supernatant fluid is decanted from the precipitate of phosphates and treated with silver nitrate (0.5 to 0.6 gram per liter). The grayish precipitate of xanthin compounds which forms is transferred to a filter and washed with water till free from chlorid; it is then suspended in water and decomposed (1) with a current of hydrogen sulphid. The liquid is filtered by decantation.

a. The filtrate is evaporated to dryness; the residue is extracted with 3 per cent. sulphuric acid to remove uric acid; the solution thus obtained, after it has been rendered alkaline

with ammonia, and filtered, if necessary, is precipitated by silver nitrate.

b. A better procedure is to concentrate the filtrate directly over the flame or on the water-bath, till the uric acid begins to crystallize out. It is then filtered, and the filtrate, after diluting somewhat with water, is rendered alkaline with ammonium hydrate in order to precipitate any remaining uric acid and phosphates. The whole is allowed to stand one or two days, then filtered, and the filtrate again precipitated with silver nitrate.

(2) The decomposition of the washed silver precipitate was made by BALKE with sodium sulphid, as in Ludwig's method. The filtrate containing the sodium compounds was decomposed with hydrochloric acid to precipitate the uric acid. The solution filtered, concentrated, and treated with ammonia. The precipitate, which according to SALOMON consists of phosphates, was found by BALKE to be urates and oxalates with no phosphates. It is filtered off, and the ammoniacal solution precipitated with silver nitrate.

The thoroughly washed precipitate of the silver xanthin compounds, now free from uric acid, and obtained by either one of the three above-mentioned methods, is dissolved in as little as possible of hot nitric acid of specific gravity 1.1, to which a little urea has been added, and the clear solution, with addition of a little silver nitrate, is set aside for twenty-four hours. The silver salt of hypoxanthin crystallizes from the solution and is filtered off. It can be purified by repeated recrystallization from hot nitric acid containing a little urea, then decomposed with hydrogen sulphid, and the filtrate, rendered alkaline with ammonium hydrate, is concentrated to a small volume. On standing, pure hypoxanthin crystallizes out.

BALKE, by digesting the hypoxanthin silver nitrate on a water-bath with dilute ammonia, converted it into hypoxanthin-silver. From the feebly ammoniacal filtrate, on standing twelve hours, the small needles of episarkin separate and can

be recrystallized from hot water. A part of the episarkin remains on the filter as a compound with two atoms of silver. Hence, on decomposition of the hypoxanthin silver with hydrogen sulphid the filtrate on concentration will give a crystalline deposit of hypoxanthin and episarkin. The two bases are then separated, as given on page 504, by dissolving in very little dilute ammonia, and then passing through the solution a current of carbonic acid, which precipitates the episarkin, probably as an ammonium salt, in small whetstone shaped needles, which, recrystallized from hot water, yield the base.

The filtrate from the silver salt of hypoxanthin on being rendered alkaline with ammonium hydrate gives a precipitate which formerly was regarded as consisting entirely of the xanthin silver compound, but which from the investigations of SALOMON has been shown to be a mixture of the salts of xanthin, paraxanthin, and heteroxanthin.

The separation of these bases is effected by the solubility of the free bases in ammonium hydrate. For this purpose the precipitate of the mixed silver salts is decomposed with hydrogen sulphid, and the filtrate, rendered ammoniacal to remove traces of phosphates and oxalates, is moderately concentrated. After standing twenty-four hours, heteroxanthin crystallizes out, partly in finely formed sheaves and tufts of needles, partly in radially striated masses. The fluid is decanted from the crust of heteroxanthin which forms in the bottom of the beaker, and after being concentrated somewhat is again allowed to stand. In this way a second crop is obtained, and this is repeated till finally the separated masses scarcely give a precipitate with sodium hydrate. All the heteroxanthin is now united and dissolved in a little hot water by the aid of sodium hydrate. After twenty-four hours the greater part of the heteroxanthin crystallizes out in bunches of crystals of sodium heteroxanthin, while a small part together with any traces of xanthin remains in solution. The crystalline mass is dried by pressure, dissolved in a little water, and the solution neutralized by addition of hydro-

ehloric acid, when the heteroxanthin separates as a pulverulent precipitate. To remove any traces of paraxanthin, dissolve in hydrochloric acid; on standing forty-eight hours the heteroxanthin salt separates, while the easily soluble salt of paraxanthin remains in solution. To obtain the pure free heteroxanthin, the hydroehloric salt is evaporated with ammonium hydrate; the well-washed residue of heteroxanthin is then dissolved in dilute ammonia, the solution filtered, evaporated slowly, and the precipitate which forms is finally washed with alcohol and ether.

The original ammoniacal mother-liquors of heteroxanthin yield on further concentration amorphous floecules of xanthin, which are removed by filtration; from the filtrate, when concentrated still more, paraxanthin crystallizes out.

Detection of xanthin, heteroxanthin, and paraxanthin, in small quantities of urine (1-5 liters or more). After the decomposition of the first ammoniacal silver precipitate with hydrogen sulphid, the filtrate is evaporated to dryness, and, in order to remove uric acid, extracted with dilute sulphuric acid (1 : 30). Any crystals, granules, or masses in the residue on evaporation are carefully removed with platinum spatula, rinsed, and treated as follows :

(1) Crystals or crystalline granules; the water solution on cooling gives typical paraxanthin crystals. A crystal is moistened with water and covered with a little sodium hydrate. The formation of a crystalline scum confirms presence of paraxanthin.

(2) Granules; the water solution on cooling gives amorphous or roundish masses. Treat some granules as under 1.

(a) A crystalline scum forms. Transfer to unglazed porcelain, wash, place in a solution of ammonium nitrate or ehlorid.

I. Typical paraxanthin crystals or bundles of long needles separate, paraxanthin.

II. Amorphous masses separate which gradually assume the roundish form. Heteroxanthin is probably indicated.

Boil all the material with a little water; treat the insoluble portion with dilute sodium hydrate, and allow it to evaporate. Twin crystals and their polariscopic behavior prove the presence of heteroxanthin.

(b) The granules dissolve easily and rapidly. Xanthin is probably present. Apply the xanthin-reaction.

(3) The substance is homogeneous and amorphous. The dried mass is rinsed with water to remove ammonium salts, dissolved in a little sodium hydrate, and set aside in a watch-glass to evaporate. The crystalline tufts are transferred to porcelain, drained, washed, and placed in an ammonium salt solution.

(a) Paraxanthin crystals separate.

(b) Amorphous masses form—probably heteroxanthin.

On converting the apparently pure xanthin, prepared as above, into the lead compound, for the purpose of decoloration, decomposing it with hydrogen sulphid, and allowing the filtrate, rendered ammoniacal, to stand over night, BALKE obtained a deposit of the crystalline modification of heteroxanthin.

Paraxanthin is obtained in colorless, glassy, generally six-sided plates, which are arranged in tufts or rosettes. From very concentrated aqueous solutions it crystallizes in long, colorless, interwoven needles, which on drying exhibit the silky lustre of tyrosin. The crystals belong to the monoclinic system, and may crystallize with, as well as without water.

For photographic illustration of the crystals, see SALOMON, 1884.

If water is present, on careful heating (110°) the crystals lose their brilliancy and become whitish and opaque, and at 120° – 130° the water is completely driven off. The conditions under which crystals containing water are formed are not known, probably by slow crystallization, whereas rapid crystallization from hot concentrated solution yields the anhydrous needles. At about 170° – 180° sublimation takes place. The melting-point is at about 284° (KOSSEL). It can be heated to 250° without melting or suffering any de-

composition, but when heated more strongly it gives off white vapors which possess a distinct iso-nitril odor, at the same time it carbonizes and takes fire. When evaporated with concentrated nitric acid, as in the ordinary xanthin-test, it gives only a slight yellow residue. On the other hand, Weidel's test, evaporation with chlorine water containing a trace of nitric acid, and then placing the dry residue into an ammoniacal atmosphere under a bell-jar, gives a beautiful rose-red color.

It is difficultly soluble in cold water (though more easily than xanthin); somewhat more readily soluble in hot water, and insoluble in ether and alcohol. It is soluble in ammonium hydrate, hydrochloric acid, and nitric acid. Its solutions are neutral in reaction.

Silver nitrate produces in nitric acid, as well as in ammoniacal solutions, a flocculent or gelatinous precipitate, which in concentrated solutions forms an almost perfect jelly-like mass. This silver precipitate is soluble in warm nitric acid, from which on cooling it separates in white crystalline tufts possessing a silky lustre. On decomposition with hydrogen sulphid the silver salt yields pure paraxanthin. Picric acid produces in the hydrochloric acid solution a precipitate consisting of densely felted yellow crystalline spangles.

It is also precipitated by phosphotungstic acid and copper acetate; mercuric chlorid when added in excess gives after some time a precipitate composed of a mass of colorless prisms, which are rather difficultly soluble in water; easily in hot water. The crystals of paraxanthin mercuric chlorid when moderately heated become opaque from loss of water of crystallization; at a higher temperature they melt, undergoing at the same time partial decomposition, and on strong heating they evolve disagreeable nauseating vapors. The aqueous solution of this mercuric double salt gives with silver nitrate an abundant precipitate of silver chlorid, which disappears on the addition of ammonium hydrate, and is replaced by the flocculent gelatinous precipitate of silver paraxanthin. The hydrochloric acid solution of paraxanthin crystallizes with

difficulty even when strongly concentrated, and on the addition of platinum chlorid it yields a well-crystallizable orange-colored paraxanthin platinochlorid. It is not precipitated by basic lead acetate nor by mercuric nitrate. Copper solutions, in the presence of reducing substances, give a flocculent milk-white precipitate, which on washing turns greenish by oxidation (BALKE, page 425). Pieric acid gives a yellow precipitate in a hydrochloric acid solution of the base.

In its behavior to the xanthin test this base resembles hypoxanthin, whereas in giving Weidel's reaction it approaches xanthin. Finally, it coincides with guanin by yielding a precipitate with pieric acid. Although it thus agrees in some of its reactions with all three of these xanthin bodies, it can, however, be easily distinguished from them by its behavior with the fixed alkalis. Sodium or potassium hydrate dissolves these bases and holds them in solution, but when added to concentrated paraxanthin solution the alkali produces a precipitate of long, glittering, crystalline spangles, which under the microscope are seen to consist of delicate rectangular, often longitudinally striated, plates which are isolated or united in tufts. The plates show double refraction. Besides these crystals there are also present hexagonal plates resembling cystin. The crystals are soluble in a little water, or on warming, but precipitate again on cooling. Paraxanthin, however, shares with heteroxanthin the property of forming a difficultly soluble compound with the fixed alkalis, but can be distinguished from the latter by neutralizing with an acid the solution of the sodium or potassium compound, when, in the case of paraxanthin, there will be obtained a precipitate of the characteristic crystals of that base; whereas heteroxanthin is obtained on similar treatment as a dense pulverulent precipitate. This reaction is not given by theophyllin.

It is interesting to observe that paraxanthin is isomeric with theobromin, theophyllin, and also with a body recently described by FISCHER as dioxy-dimethyl-purpurin. In its composition it is, therefore, a dimethyl- or an ethyl xanthin.

The physiological action of paraxanthin has been studied by SALOMON. Injections into the muscles of 1–2 mg. produced almost at once a *rigor mortis*-like condition of the muscles affected, with diminished reflex excitability without previous increase; 6–8 mg. introduced into the lymph sac bring on a gradual loss of voluntary motion as well as of reflex excitability; the rigor is more marked in the anterior extremities, which have a wooden or waxy consistency. Dyspnoea is likewise an early symptom, but as soon as rigor sets in the respirations drop far below the normal, and may be absent for several minutes. At times the lungs are enormously dilated, same as with theobromin. The heart's action is intact till the very last. In mice the reflexes are increased almost to a tetanus. An injection of 0.2 g. in a 500 g. guinea-pig produced convulsions and death in a half an hour. The same dose introduced into the vein of a rabbit had no effect. The lethal dose for frogs, subcutaneously, was found to be 0.15–0.2 per cent. of the body-weight—somewhat lower than that of theobromin and xanthin. The action of these three bases is very similar. They produce in common the slow-creeping movements, followed by cessation of spontaneous muscle-action, complete loss of reflex excitability without a previous rise, and the heart's action is not affected till in the latest stages.

CARNIN, $C_7H_8N_4O_3$, was isolated in 1871 from American meat-extract by WEIDEL, but has not been obtained from muscle-tissue itself. BALKE, however, has isolated it from fresh horse-meat extract. It has also been obtained from yeast liquors by SCHÜTZENBERGER, and from urine by PORCHET. SALOMON (1893) obtained a body resembling carnin from leukaemic urine. It can be separated from the meat-extract by the following method originally employed by WEIDEL: The extract is dissolved in six or seven parts of warm water, then concentrated baryta-water is added, avoiding, however, an excess. The filtrate is precipitated by basic lead acetate. The precipitate is collected, thoroughly washed and pressed, and finally it is repeatedly extracted with a large quantity of

boiling water. The carnin lead salt is thus dissolved out; the filtrate, after removal of the lead by hydrogen sulphid, is evaporated to a small volume. The concentrated solution thus obtained is treated with silver nitrate, which gives a precipitate of silver chlorid and of the silver salt of carnin. By treatment with ammonium hydrate the silver chlorid can be completely removed from the precipitate, whereas the silver compound of carnin is insoluble in that reagent. To obtain pure carnin the silver salt is decomposed with hydrogen sulphid, and the filtrate, after purification by bone-black, is evaporated to crystallization. According to WEIDEL, carnin forms about 1 per cent. of the meat extract. KEMMERICH (1893) has found only one-quarter of 1 per cent., or even less. The amount, therefore, varies considerably, and may be very small in the fresh extract.

For the separation of carnin from the meat-extract by the copper method, see page 477.

Carnin forms white crystalline masses, which on drying become loose and chalk-like. It is very difficultly soluble in cold water, easily and completely in boiling water, and recrystallizes on cooling. It is insoluble in alcohol and ether. The taste is decidedly bitter, and the reaction is neutral. The base is not precipitated by neutral lead acetate, but is precipitated by the basic salt as a flocculent white precipitate soluble in boiling water. On heating carnin decomposes and takes fire, and at the same time gives off a peculiar odor. It crystallizes with one molecule of water, which it loses at 100° – 110° .

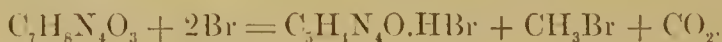
The hydrochlorid, $C_7H_8N_4O_3.HCl$, forms pretty prisms (KEMMERICH), and decomposes on heating with concentrated hydrochloric acid.

The platinochlorid, $C_7H_8N_4O_3.HCl.PtCl_5$, forms a fine, sandy, golden-yellow powder.

With silver nitrate carnin unites to form a white flocculent precipitate, insoluble in nitric acid or in ammonium hydrate. Its formula corresponds to $2(C_7H_7AgN_4O_3) + AgNO_3$.

With copper solution and reducing agent it gives a yellow precipitate which on washing becomes grass-green (BALKE).

Carnin is not affected by prolonged boiling with concentrated barium hydrate. Bromine-water decomposes it with the evolution of gas and the formation of hypoxanthin. This change takes place according to the following equation :



A similar decomposition into hypoxanthin is brought about by the action of nitric acid, though in this case oxalic acid and a yellow body are formed. When carnin is evaporated with chlorine-water containing a little nitric acid the residue, on contact with ammonia, gives a rose-red color (murexid-test). This is due, according to WEIDEL, to the formation of hypoxanthin, but it has since been shown that the latter base does not give this reaction, and hence it is due to the production of xanthin, or some similar body.

The physiological action of carnin has been examined somewhat by BRÜCKE, and according to him it is not very poisonous. The only effect observed, when taken internally, was a fluctuation in the rate of the heart-beat, though even this was by no means definite in its nature.

EPISARKIN, $\text{C}_4\text{H}_6\text{N}_3\text{O}$. This new base was isolated by BALKE in 1893 from urine. From about 1600 liters of urine only about 0.4 g. was obtained. It accompanied the hypoxanthin silver nitrate (page 496). The mixture on digestion with dilute ammonia on a water-bath is changed into hypoxanthin-silver, whereas episarkin, in part, passed into solution. The solution was filtered, and the slightly ammoniacal filtrate on standing for twelve hours gave a crop of small needles of episarkin. The hypoxanthin-silver still contained an admixture of the new base, as was found by decomposition with hydrogen sulphid and concentration. The crystals are more difficultly soluble in water, and their crystalline form is distinctly different from that of hypoxanthin. A separation can best be accomplished by dissolving the mixture

of the two bases in as little dilute ammonia as possible, then on saturating this solution with carbonic acid small whetstone-shaped needles separate, which recrystallized from hot water yield the small prisms or needles. These may attain a length of 1 cm. and may be grouped in bunches. They are white and glassy, and when dry form a felt-like mass. The crystals are permanent and do not effloresce. With the xanthin-reaction, evaporation with concentrated nitric acid, it gives a yellow residue, which with sodium hydrate becomes paler. On evaporation with nitric acid and chlorine-water (Weidel's reaction), it gives a white residue unaltered by ammonia. When evaporated on a water-bath with hydrochloric acid and potassium chlorate it leaves a white residue, which becomes colored an intense violet by ammonia. This reaction certainly shows a relationship of episarkin to the xanthin bases, and should be compared with KOSSEL's bromine-reaction for adenin and hypoxanthin (page 424).

It is difficultly soluble in hot water; almost wholly insoluble in cold water (1 : 13,000). It is readily soluble in dilute hydrochloric acid, from which, on evaporation, the hydrochlorid crystallizes in easily soluble, pretty needles. The base does not yield an insoluble compound with sodium hydrate, though it is possible that the whetstone-shaped crystals mentioned above represent an ammonium salt. With silver nitrate it gives a precipitate which is insoluble in nitric acid, easily soluble in ammonia. In other respects the salt resembles that of hypoxanthin. Thus, by boiling with nitric acid (1.1 sp. g.) a crystalline silver nitrate compound forms which probably contains one atom of silver, and on digestion with dilute ammonia yields a silver compound with two atoms of silver—since a part of the episarkin passes into the filtrate and crystallizes out on cooling. The base gives white precipitates with phosphotungstic acid, mercuric chlorid, and ammoniacal basic lead acetate.

It is distinguished from adenin and hypoxanthin by its almost complete insolubility in cold water; from the latter by not being precipitated by picric acid, and by not giving the

characteristic ruby-red coloration in the alkalized filtrate after reduction with zinc and hydrochloric acid (page 424). From xanthin it is distinguished by the absence of the xanthin reaction; from hetero- and paraxanthin by the absence of the insoluble sodium compound; from guanine and adenine by not clouding at 53°, and by the picric acid reaction.

SALOMON has since called attention to the fact that he had met with a compound resembling episarkin on several occasions. Thus, in 1884, from hog's urine (0.02 g. in 5½ liters; in 1888, from cow's urine (0.05 g. in 60 liters); and, 1892 and 1893, in two cases of leukæmic urine (0.02 g. in 11 liters, 0.13 g. in 35 liters). The specimen from the hog urine gave the xanthin-reaction when heated over a free flame, but not when heated on a water-bath. Furthermore, in all three cases it gave an insoluble precipitate of yellow rosette-shaped crystals with picric acid; whereas BALKE's episarkin gives no precipitate. SALOMON isolated it from the leukæmic urine as follows: After removal of the phosphates by ammonia (see page 495), the urine was precipitated by silver nitrate, the precipitate decomposed by hydrogen sulphid, the filtrate evaporated to dryness and digested with 3 per cent. sulphuric acid. The filtrate was now rendered slightly ammoniacal and after a few minutes filtered. The filtrate from this on standing twenty-four hours gave a deposit of yellow-colored crystals. These were filtered off and the ammonia expelled from the filtrate, which contains xanthin bodies. From the hypoxanthin fraction of these an additional yield of crystals was secured. The combined crystals were then dissolved in dilute hydrochloric acid, filtered; the filtrate saturated with ammonia gave a dense white precipitate which was brought into solution again by dilution and heat. The base then separated slowly in long colorless prisms, which on the filter appear as a felt-like, silky mass.

KRÜGER, in 1895, from tea-extract isolated a base resembling somewhat episarkin. It differs, however, from the latter in giving with picric acid a very fine crystalline compound. It is more soluble in water, and gives different color-reactions.

Nothing definite can be stated regarding the constitution of episkarkin. The formula as given by BALKE is open to the objection that the sum of the hydrogen and nitrogen atoms is an odd number. Changing for this reason the number of hydrogen atoms to seven, we would then have a compound, $C_4H_7N_3O$, distinguished from hypoxanthin by having three atoms of H more and a carbon and nitrogen atom less. Inasmuch as it gives the modified xanthin-reaction an alloxan group must be present, and in that case it can only be derived from the hypoxanthin formula 3. Whether its structure

would then correspond to
$$\begin{array}{c} \text{NH}-\text{CH}_2 \\ | \quad \quad || \\ \text{CO} \quad \text{C}=\text{NH}_2 \\ | \quad \quad | \\ \text{NH}-\text{CH}_2 \end{array}$$
 remains to be ascertained.

EPIGUANIN, $C_{10}H_{13}N_9O_2$, is a base obtained by KRÜGER and WULFF, in 1893, from urine. It forms silky needles and is different from the base isolated from tea-extract (page 506). Another base was present in small quantity in the same urine.

CYTOSIN, $C_{21}H_{30}N_{16}O_4 + 5H_2O$, was obtained by KOSSEL and NEUMANN by the decomposition of adenylie acid (from thymus glands) by heating with 20 per cent. sulphuric acid in sealed tube at 150° ; also by the action of water at 170° . The yield is about 2 per cent. It forms rectangular plates, often with blunted corners. On slow separation the crystals may attain a length of a centimetre. The water of crystallization is expelled at 100° . It is easily soluble in hot water, from which it separates on cooling; difficultly soluble in alcohol; insoluble in ether.

It forms well-crystallized salts. Thus, the sulphate forms needles; the chlorid is easily soluble and appears in prisms. The nitrate, platinochlorid, and amrochlorid likewise crystallize easily. A brick-red precipitate forms in even very dilute acidulated solution by the addition of potassium-bismuth iodid. Silver nitrate produces a precipitate which is in-

creased by addition of a little ammonia, but dissolves gradually by an excess, and on warming the ammoniacal solution it dissolves completely, but reappears in crystalline form on cooling.

The picrate, $C_{21}H_{30}H_{16}O_4 \cdot 2C_6H_3N_3O_7$, is difficultly soluble and crystallizes in yellow needles.

A BASE, $C_4H_5N_5O$, was obtained by GAUTIER from fresh muscle tissue of beef, according to the method given on page 516, and on account of a resemblance in some of its properties with xanthin he named it pseudoxanthin. This name is very inappropriate, not only because it differs so much in its empirical formula from that of xanthin, $C_5H_4N_4O_2$, but also because the term pseudoxanthin has already been applied by SCHULTZEN and FILEHNE to a body isomeric with xanthin, which was obtained by the action of sulphuric acid on uric acid.

The free base forms a light yellow powder, slightly soluble in cold water, soluble in weak alkali and in hydrochloric acid. The hydrochlorid is very soluble, and it forms stellate prisms with curved faces, which resemble the corresponding salt of hypoxanthin, and to some extent, also, the whetstone-shaped crystals of uric acid.

Like xanthin, its aqueous solution is precipitated in the cold by mercuric chlorid, silver nitrate, and by ammoniacal lead acetate, but not by normal lead acetate. On evaporation with nitric acid, the residue gives, on contact with potassium hydrate, as in the case of xanthin, a beautiful orange-red coloration (xanthin-reaction). It differs from xanthin, not only in its empirical composition, but also in its greater solubility and in its crystalline form. It is possible that this base, on account of its great resemblance to xanthin, may have been mistaken, at different times, for that compound. It will be seen from the formula that it differs from xanthin apparently by an NH group replacing a CO group.

GERONTIN, $C_5H_{11}N_2$, is a new base which was isolated by GRANDIS in 1890. It has been repeatedly observed in the

form of peculiar crystals found in the cell nuclei in the liver and kidneys, particularly of old dogs. The free base is an isomer of cadaverin, etc., and resembles it somewhat. It crystallizes in needles which are readily soluble in water and alcohol; possesses a strongly alkaline reaction, and yields the ordinary alkaloidal reactions.

The hydrochlorid forms prismatic crystals, which are deliquescent and easily soluble in alcohol.

The platinumochlorid, $C_5H_{11}N_2 \cdot 2HCl.PtCl_6$, is soluble in water and crystallizes in spindle-shaped needles, arranged in rosettes. It decomposes at 115° .

The gold salt forms small needles, and is easily soluble in water and alcohol.

It combines with one molecule of mercuric chlorid to form deliquescent cubes or rectangular prisms containing two molecules of water of crystallization. It decomposes above 100° . This distinguishes it from cadaverin, which combines with three to four molecules of mercuric chlorid. The crystals observed in the liver are probably the phosphate.

The new base also yields a benzoyl compound which melts at 175° – 176° .

Physiological Action.—It seems to exert a paralyzing action upon the nerve-centres and heart-ganglia, and leaves the nerves and muscles unaffected. 0.5 mg. kills 10 g. frogs.

SPERMIN, C_2H_5N , or $C_5H_{11}N_2$, is the basic substance obtained by SCHREINER (1878) from semen, calf's heart, calf's liver, bull's testicles, from the organs of leucocythæmies, and also from the surface of anatomical specimens kept under alcohol. POEHL has found it in the testes, ovaries, prostate, thyroid gland, pancreas, and spleen. In 1888 KUNZ reported the presence of a non-poisonous base, C_2H_5N , spermin or ethylencimid in cholera cultures. In this case it occurs, then, as a ptomain. A confirmation of the identity of the two bases is necessary. Previous to this, however, it had been known for a long time under the name of "Charcot-Neumann or Leyden crystals," which are the phosphate of spermin. These

peculiarly shaped crystals have been found in the sputa of a case of emphysema with catarrh, in the bronchial discharges in acute bronchitis, as well as in sputa of chronic bronchitis, in the blood, spleen, etc., of leucoeythæmics and anæmics, and in the normal marrow of human bones, as well as in human semen, also in nasal secretions and in feces. Altogether it seems to have a very wide distribution, especially in certain diseases, as in leucoeythæmia.

It can be prepared from fresh human semen in the following manner: The semen is washed out of linen by a little warm water, evaporated to dryness, boiled with alcohol, and the insoluble portion is allowed to subside by standing some hours. The precipitate is filtered off, washed, and dried at 100° . This residue, containing the spermin phosphate, is triturated, and then extracted with warm ammoniacal water. From this solution, on slow evaporation, the phosphate crystallizes in its peculiar-shaped crystals.

The free base is obtained, on decomposing the phosphate with baryta and evaporating the filtrate, as a colorless liquid, which, on cooling, crystallizes. From alcohol it crystallizes in wavellite-shaped crystals, which readily absorb water and carbonic acid from the atmosphere. They are readily soluble in water and in absolute alcohol, almost insoluble in ether, and possess a strongly alkaline reaction. When heated on platinum foil it gives off thick, white fumes and a weak ammoniacal odor. With potassium-bismuth iodid it yields orange-colored crystalline floccules, which under the microscope appear as long, sharp, plumose needles—distinction from diethylenediamin. The aqueous solution of the base is precipitated by phosphomolybdic and phosphotungstic acids, tannic acid, gold and platinum chlorids. It cannot be volatilized from alkaline solution by steam without undergoing decomposition (MAJERT and SCHMIDT). It is not poisonous.

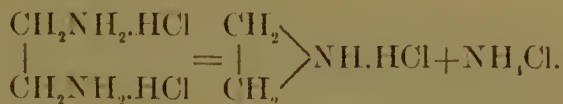
The hydrochlorid, $C_2H_5N.HCl$ (?), crystallizes in six-sided prisms, united in tufts, and is extremely soluble in water, almost insoluble in absolute alcohol and ether.

The aurochlorid, $C_2H_5N.HCl.AuCl_3$ (?), forms shining,

golden-yellow, irregular plates, and when freshly precipitated it is easily soluble in water, alcohol, and ether, but the dried salt is incompletely soluble in water. The aqueous solution, treated with magnesia, gives off a sperm-like odor. The platinochlorid crystallizes in prisms.

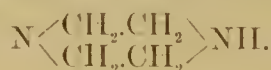
The phosphate, $(C_2H_5N)_2.H_3PO_4 + 3H_2O$ (?), forms prisms and slender double pyramids arranged in rosettes. It is difficultly soluble in hot water, insoluble in alcohol, easily soluble in dilute acids, alkalis, and alkali carbonates. It melts with decomposition at about 170° . It is probable that the above formula does not represent the salt as found, and from theoretical considerations LADENBURG was inclined to think that Schreiner's phosphate had the composition $(C_2H_5NH)_4Ca(PO_4)_2$.

LADENBURG and ABEL prepared in 1888 a compound, ethyleneimin, which was first supposed to be isomeric with spermin. The reaction whereby it is prepared is similar to the one by which LADENBURG effected the synthesis of piperidin. Ethylenediamin hydrochlorid is subjected to dry distillation, when it decomposes into ammonium chlorid and the hydrochlorid of the new base. The reaction was supposed to be represented by the equation :



Since then LADENBURG has shown that the boiling-point of this compound did not agree with what it should be theoretically, if represented by the above formula. A determination of the vapor density showed that the molecular weight was twice that corresponding to the formula given, and hence was $C_4H_{10}N_2$. MAJERT and SCHMIDT assuming spermin to be ethyleneimin, as was apparently shown by LADENBURG and ABEL's investigation, attempted to prepare the latter on a manufacturing scale with the expectation that it might be used as a substitute for Brown-Séquard's testicular fluid. They were soon able to show, however, that ethyleneimin did

not possess the composition assigned to it, but that it was identical with HOFMANN's diethylenediamin (piperazin),



This was soon confirmed by HOFMANN and by LADENBURG. Spermin was then assumed to be identical with piperazin, but recently (1891) MAJERT and SCHMIDT compared some spermin from SCHREINER with their own piperazin and found the two bases to be distinct, especially with reference to the phosphates and the potassium-bismuth iodid precipitates. POEHL confirmed the difference between spermin and piperazin.

About the same time (1891) POEHL announced that the composition of spermin was more complex than what it had been hitherto supposed to be. He ascribed to it the formula $\text{C}_{16}\text{H}_{26}\text{N}_4$. The formula of the platinum salt corresponded to $\text{C}_{10}\text{H}_{26}\text{N}_4 \cdot 4\text{HCl} \cdot 2\text{PtCl}_4$; and that of the gold salt was represented by $\text{C}_{10}\text{H}_{26}\text{N}_4 \cdot 4\text{HCl} \cdot 4\text{AuCl}_3$. Later he gave the formula $\text{C}_5\text{H}_{11}\text{N}_2$.

From this it would appear that spermin is essentially distinct from piperazin. The composition and structure of this interesting base must therefore be considered as not settled.

According to POEHL, it acts as a tonic on the entire nervous system.

The nuclein of the spawn of salmon has been found by MIESCHER to exist in a salt-like combination with a basic substance, to which he applied the name protamin. PICCARD has found it in the same source, together with hypoxanthin and guamin, but no xanthin. The formula assigned to this base is quite complex, and cannot be considered as definitely settled. It would seem from the copper-reaction to be related in some way with the xanthin bases. Analysis of the platinochlorid gave: $\text{Pt} = 24.64$, $\text{Cl} = 26.45$, $\text{N} = 15.03$, $\text{C} = 22.80$, $\text{H} = 4.15$, $\text{O} = 6.93$. The hydrochlorid forms an amorphous, hygroscopic, sticky mass. It does not give the xanthin-reaction. With sodium hydrate and copper sulphate it gives the biuret-reaction; the xanthoproteic and Millon's tests are not given. The alkaline copper solution with hy-

droxylamin hydrochlorid gives a clay-yellow precipitate and the violet solution becomes colorless. The precipitate on exposure to air becomes bluish-green; on decomposition it yields pure protamin hydrochlorid (BALKE).

LEUCOMAINS OF THE CREATININ GROUP.

The knowledge of the formation of basic substances (ptomaines) during the putrefaction of nitrogenous organic matter led to a series of investigations having for their object the isolation of alkaloidal bodies, if such existed, from the normal living tissues of the organism. A number of compounds possessing alkaloidal properties, such as the xanthin-derivatives, already described, had been known for a long time, although their physiological relation to the animal economy was little, if at all, understood. GUARESCHI and MOSSO, in the course of their researches on ptomaines, were among the first to direct their attention to the possible presence of ptomain-like bodies in fresh tissues. They obtained in those cases where the extraction was carried on without use of acids only very minute traces of an alkaloidal body (possibly cholin), and an inert substance, methyl-hydantoin, which, although it can scarcely be classed as a basic compound, is closely related to creatin, and for this reason will be described at the end of this section. Other Italian chemists, as PATERNO and SPICA and MARINO-ZUCO, had also shown that the normal fluids and tissues of the body were capable of yielding substances alkaloidal in nature, and these were regarded by them as identical with, or similar to, the ptomaines of SELMI.

Arginin, $C_6H_{11}N_4O_2$, is a base obtained by SCHULZE from the conglutin of lupin sprouts, and according to him it is related to creatinin and possibly to the leucomains of GAUTIER. A compound having the same composition and probably identical with arginin has been obtained by HEDIN by the decomposition of horn substance with hydrochloric acid and stannous chlorid. SCHULZE and LUKIERNIK have demonstrated the interesting fact that arginin on heating with baryta yields urea. Arginin is present in the sprouts of lupin

and of gourd, but not in the sprouts of *Vicia sativa*, where it appears to be replaced by guanidin.

Lysatin, $C_6H_{13}N_3O_2$, and lysatinin, $C_6H_{11}N_3O$, are analogous bases, obtained by DRECHSEL from casein (page 367). These two bases are also obtained by decomposition with hydrochloric acid, of gelatin (E. FISCHER); of other proteids (SIEGFRIED); and of horn substance (HEDIN).

Lysatinin has also been isolated from the elastic substance of the aorta by heating with hydrochloric acid and stannous chlorid (SCHWARZ). HEDIN (1895) pointed out that lysatinin is probably a mixture of lysin (lysatin) and arginin.

The various tissues of the body on boiling with hydrochloric acid yield variable amounts of arginin. Thus, horn yields at least 2.25 per cent.; gelatin, 2.6; conglutin, 2.75; albumin from yolk, 2.3; albumin from egg, 0.8; dry blood-serum, 0.7; casein, 0.25 per cent. (HEDIN). SCHULZE found the dried cotyledons of lupines to yield 4 per cent.

These three bodies can properly be looked upon as important sources of the nitrogenous bases found in animals and plants. LIEBREICH, in 1869, discovered in normal urine an oxidation-product of cholin, probably identical with betain (page 376), and POUCHET, in 1880, announced the presence in the same secretion of allantoin, carnin, and an alkaloidal base, which, however, was not obtained at that time in sufficient quantity to permit a determination of its character. Subsequently he succeeded in isolating this base as well as another closely related body, both of which will be described in their proper place. GAUTIER devoted a number of years to the study of the leucomaïns occurring in fresh muscle tissue, and he succeeded in isolating several new compounds.

A number of these substances are credited with possessing an intensely poisonous action, and if such is the case, it is very evident that any undue accumulation of such bases in the system, resulting from an interference in the elimination, may give rise to serious disturbances. The amount of these substances present in the daily yield of the urine is very small—

so small, indeed, that we must rather look upon this small quantity as having escaped oxidation in the body. It is well known that the living tissues possess an enormous oxidizing and reducing power, and, according to GAUTIER, there is constantly going on in the normal tissues of the body a cycle of changes—the formation of leucomains and their subsequent destruction by oxidation, before they have accumulated in sufficient quantity to produce poisonous effects.

The following method was employed by GAUTIER in his study of the leucomains of muscle tissue: The finely divided fresh beef-meat or the Liebig's meat-extract is treated with twice its weight of water, containing 0.25 gram of oxalic acid, and one to two c.c. of commercial peroxid of hydrogen per liter. The purpose of these precautions is to prevent fermentation. At the end of twenty-four hours the liquid is raised to the boiling-point, then filtered through linen, and the residue is thoroughly squeezed. The filtrate is again raised to the boiling-point in order to coagulate any remaining albumin, and finally filtered through paper. The clear liquid thus obtained is evaporated in a vacuum at a temperature not exceeding 50°, and the acid syrupy residue is extracted with 99 per cent. alcohol; the alcoholic extract is in turn evaporated in a vacuum, and the residue taken up with warm alcohol of the same strength. The filtered alcoholic solution is set aside for twenty-four hours, and any deposit which forms is removed by filtration; ether (65°) is then added as long as a precipitate continues to form, and the whole is again allowed to stand for twenty-four hours. The ether-alcoholic filtrate from this precipitate is evaporated first on the water-bath, and finally in a vacuum; the slight residue obtained contains a small quantity of basic substances possessing an odor of hawthorn.

The syrupy precipitate produced by the ether partially crystallizes on standing; a little absolute ether is then added, and after standing several days more the liquid is separated by means of an aspirator from the deposit of crystals (A). These are first washed with 99 per cent. alcohol, and then extracted with boiling 95 per cent. alcohol. The alcoholic

solution, concentrated by evaporation, gives, on cooling, a deposit of lemon-yellow crystals of xantho-creatinin (B), from the mother-liquor of which there separates a crop of new crystals (C). The residue of the crystals (A) left after treatment with the boiling 95 per cent. alcohol is extracted with boiling water, which afterward gives a slight deposit of yellowish white crystals of amphi-creatin (D). The aqueous mother-liquor on concentration yields another deposit of orange-colored crystals of cruso-creatinin (E). GAUTIER has, furthermore, separated three other bases from the mother-liquors of the crystals obtained as above. Thus, a base which he named pseudoxanthin is stated to have been obtained by evaporating the alcoholic mother-liquors of B, D, E (?) in a vacuum, taking up the residue with water, and precipitating the hot solution with copper acetate. The precipitate is decomposed with hydrogen sulphid, and the aqueous solution, filtered while boiling-hot, yields a deposit of a sulphur-yellow powder of pseudoxanthin. Thus, by the use of alcohol, ether, and water, GAUTIER, according to his statement, has succeeded in obtaining a sharp separation between these bases. The importance of the subject is such as to require not only confirmation of the results arrived at by GAUTIER, but also a more detailed and exact study of the chemical and physiological behavior of these bodies.

The following method was employed by GAUTIER and LANDI in 1892 in their study of the changes in meat. The meat-extract was concentrated in a vacuum to one-eighth its volume, then cooled, precipitated with neutral lead acetate, filtered, and after washing the precipitate, the filtrate was again concentrated to one-half its volume, and the lead removed by hydrogen sulphid. The filtrate was again concentrated to one half its bulk and dialyzed. The bases are present in the dialysate. The dialyzed portion therefore was concentrated, acidulated with nitric acid, and precipitated with phosphomolybdic acid. The precipitate is collected and washed at once with very dilute nitric acid, then with water. It is then boiled with neutral lead acetate; the bases and the greater

part of the xanthin and carnin pass into solution. After removal of the lead, the filtrate is evaporated in a vacuum, then extracted with alcohol. The residue is examined for:

Bases A. It is treated with dilute ammonia; this dissolves xanthin, hypoxanthin, gnanin, carnin, etc., whereas creatin, etc., are insoluble. The ammonia is allowed to evaporate, and hence adenin and guanin separate out. Hypoxanthin and xanthin remain in solution.

Bases B. The alcoholic filtrate from above residue is neutralized, concentrated, and treated with mercuric chlorid. The mercury precipitate is washed, decomposed with hydrogen sulphid, the solution filtered, and the filtrate is precipitated with copper acetate:

(1) In the cold—acids of the carbopyridic series, which are crystalline and give crystalline platinochlorids.

(2) In boiling solution—xanthin bases.

(3) The portion not precipitated by cold or hot copper acetate is the most important. The copper is removed with hydrogen sulphid, the filtrate evaporated to dryness, and extracted with alcohol—guanin, creatin, neurin, chlorin, butylenediamins, etc., neuridin, ethylenediamin; hydroxyridin bases and homologues, and bases that give pyrrol on distillation with lime; all are very poisonous.

Bases C. The mercuric chlorid filtrate is concentrated to drive off the alcohol and the mercury removed with hydrogen sulphid. Lead acetate is added, the liquid filtered, and after removal of the lead is evaporated to dryness and extracted with dilute alcohol—the residue was creatin; the filtrate contained oxy-ethyleneamin, methyl-guanidin, etc. Almost all of these are poisonous. They are less abundant than the others.

(a) Xanthin bases. Exist in minute amount in meat and are not toxic.

(b) Carbopyridic bases. Likewise present only in small amount. They produce stupefaction in animals, but otherwise are not dangerous.

(c) Neurin and hydroxyrrolic bases. They are the most

abundant leucomains in meat, and are the most poisonous. Minute doses in mice produce dyspnoea, spasmodic movements of the extremities, bristling of the hair, paralysis, tetanic convulsions, and death.

(d) Creatin bases. These produce in mice vomiting, diarrhoea, tetanic convulsions, followed by paralysis of the extremities.

To the physiological chemist these substances are of especial interest because of the possible relation which they bear to the formation of creatin and creatinin in the muscles. It will be seen that in the leucomains of this group, as well as in those of the uric acid group, hydrocyanic acid plays a very important part in the molecular structure of these bases. Just what the function of this cyanogen group is, so far as the vital activity of the tissues is concerned, we know very little, though recent investigations seem to show that the seat of the cyanogen formation lies within the nucleated cell, and is intimately connected with the functions of the nuclein molecule.

CRUSO-CREATININ, $C_6H_8N_4O$, forms orange-yellow crystals which are slightly alkaline in reaction and possess a somewhat bitter taste. It yields a soluble, non-deliquescent hydrochlorid crystallizing in bundles of needles; also a soluble platinochlorid which forms tufts of beautiful, slender prisms. The aurochlorid is obtained as slightly soluble, crystalline grains, and, like the platinum double salt, is partially decomposed on heating. It is not precipitated by acetate of zinc or by mercuric nitrate, but is precipitated in the cold by solutions of alum. Zinc chlorid produces in somewhat concentrated solutions a pulverulent precipitate which dissolves on heating, and recrystallizes again on cooling. Like xanthocreatinin, it is not thrown out of solution by oxalic or nitric acid, and is thus distinguished from urea and guanidin; nor is it precipitated by acetate of copper—a distinction from xanthin-derivatives. Mercuric chlorid produces an abundant flocculent precipitate which on heating partially dis-

solves, decomposing at the same time. Sodium phosphomolybdate gives a heavy yellow precipitate, whereas potassium mercurio-chlorid and iodine in potassium iodid have no effect. Potassium ferricyanid is not reduced. This base differs in its composition from creatinin by HCN, the elements of hydrocyanic acid, but in its crystalline form and alkaline reaction, and some other properties, it would seem to be closely related to this latter substance. Because of this apparent relationship and its golden-yellow color, GAUTIER named it *cruso-creatinin*.

XANTHO-CREATININ, $C_5H_{10}N_4O$, is the most abundant of muscle-leucomains. It crystallizes in sulphur-yellow, thin spangles, consisting of nearly rectangular plates which resemble somewhat those of cholesterin. It is soft and talc-like to the touch; possesses a slightly bitter taste, and when dissolved in boiling alcohol it gives off the odor of acetamid, though ordinarily in the cold it has a slight cadaverie odor. When heated, the substance evolves an odor of roast meat, carbonizes in part, and yields ammonia and methylamin. The crystals are amphoteric in reaction, are soluble in cold water, and can be recrystallized from boiling 99 per cent. alcohol.

It forms a hydrochlorid crystallizing in plumose needles, and a very soluble platinochlorid; the aurochlorid crystallizes with difficulty. Like creatinin, it is precipitated by zinc chlorid; the yellowish-white precipitate dissolves with partial dissociation on warming, and on cooling separates as isolated or stellate groups of fine needles which possess the composition $(C_5H_{10}N_4O)_2ZnCl_2$. Silver nitrate throws down, in the cold, a flocculent precipitate which likewise dissolves on heating, and recrystallizes in needles. Mercuric chlorid produces a yellowish-white precipitate. It is not precipitated by oxalic or nitric acid, nor by potassium-mercuric chlorid, or iodine in potassium iodid. Tannin produces in time a slight turbidity, while sodium phosphomolybdate gives a heavy yellowish precipitate. This base is distinguished from the

members of the uric acid group by not giving a precipitate with copper acetate, not even on heating.

On gentle oxidation with potassium permanganate it is converted into a black substance insoluble in acids and alkalis, and resembling azulmic acid. By treatment with recently precipitated mercuric oxid it yields a substance which can be recrystallized from boiling 93 per cent. alcohol in needles which possess a slight alkaline reaction, and forms a slightly soluble, crystalline platinochlorid. This new substance is precipitated from alcoholic solution, by the addition of ether, as a mass of beautiful, white silky needles resembling caffen. These crystals melt at 174° ; caffen melts at 178° .

Xantho-creatin, given in fairly large doses, is poisonous, producing in animals depression, somnolence, and extreme fatigue, accompanied by frequent defecation and vomiting. In its general properties this base resembles creatin very much, and it was on account of this resemblance and its yellow color that it was named xantho-creatinin. This relation becomes especially evident since this base appears in the physiologically active muscle at the same time with creatinin, constituting sometimes one-tenth of the creatinin present. MONARI has found this base in the aqueous extract of the muscles of an exhausted dog, and also in the urine of soldiers tired by several hours' march. He also demonstrated its presence in the urine of a dog after previous injection of creatinin. STADTHAGEN was not able to isolate this base from his urine after prolonged muscular exercise, and arrived at the conclusion that it does not occur in the urine, and that MONARI's base was an impure creatinin. COLASANTI, in 1884, and again in 1891, isolated from lion's urine by Neubauer's zinc-chlorid method for creatinin the latter compound and a yellow body which crystallized as canary-yellow, small, opaque scales, or as small, orange-yellow, granular masses composed of needle-shaped crystals. This he considers to be xantho-creatinin, derived from the large excess of creatin in the food.

AMPHI-CREATIN, $C_9H_{19}N_7O_4$, is slightly soluble, and crystallizes from boiling water in yellowish-white oblique prisms, which possess, if any, a slightly bitter taste. When heated to 100° it decrepitates somewhat, and at 110° it becomes opaque white. Potassium hydrate does not decompose it in the cold. Although a weak base, it combines to form salts just as the preceding members of this group. The hydrochlorid is crystalline, and is not deliquescent; the platinochlorid forms rhombic plates, which are soluble in water, but are insoluble in absolute alcohol; the aurochlorid crystallizes in easily soluble, very small, microscopic crystals, which are tetrahedral to hexahedral in their habit. It is not precipitated by copper acetate or by mercuric chlorid; nor does it give the murexid test, or the xanthin reaction. Sodium phosphomolybdate produces a yellow, pulverulent precipitate. In its properties it resembles creatin, and indeed GAUTIER thinks it may be possibly a combination of creatin, $C_4H_9N_3O_2$, and a base $C_5H_{10}N_4O_2$, which, it will be seen, differs from the former only by a HCN group. This second compound, if it really exists, has an analogy in cruso-creatinin, the relation of which to creatinin may be expressed by the equation:



CRUSO-CREATININ. CREATININ.

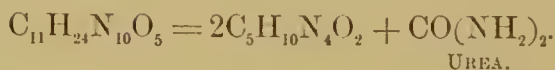
In a similar manner, amphi-creatin may be regarded as



AMPHI-CREATIN. CREATIN.

A BASE, $C_{11}H_{24}N_{10}O_5$, was isolated by GAUTIER from the mother liquors of xantho-creatinin. It crystallizes in colorless or yellowish, thin, apparently rectangular plates, which are tasteless, and possess an amphoteric reaction. The hydrochlorid forms bundles of fine needles; the sulphate yields a confused mass of needles; the platinochlorid is soluble, non-deliquescent, and crystalline. When heated with water in a sealed tube at 180° – 200° it gives off ammonia and carbonic acid, and is converted into a new base, which, however, has

not been studied. This reaction may be expressed by the equation:



The urea which at first forms is, in turn, decomposed, thus:



It is to be observed that this base differs in composition from the following one by HCN, the hydrocyanic acid molecule.

A BASE, $\text{C}_{12}\text{H}_{25}\text{N}_{11}\text{O}_5$, was obtained from the mother-liquors of eruso-creatinin, and forms rectangular silky plates, resembling those of the preceding base and of xantho-creatinin. It forms crystallizable salts.

These complex bases will require further study in order to elucidate their physiology, and the possible connection which they may have with the formation of urea and of the creatinin-derivatives already described.



This substance was obtained by GUARESCHI and MOSO (1883), by extracting fresh meat with 1-1.5 volumes of water (without addition of acid), for two hours at 50°-60°. The aqueous extract was evaporated on a water-bath and the residue was extracted with 95 per cent. alcohol. This alcoholic solution, after the alcohol was driven off, was taken up in water, filtered, and the aqueous solution was first extracted with ether, then rendered alkaline with ammonia, and again extracted with ether. The alkaline ether-extract gave on evaporation a white crystalline residue of methyl-hydantoin. The amount of this substance present in flesh appears to be quite variable, since, at times, none whatever can be extracted. ALBERTONI has isolated it from dog's flesh. Previous to its discovery in flesh by GUARESCHI and MOSO, it was known for a long time as a decomposition-product of various nitrogenous bases of the body. Thus, NEUBAUER prepared it by heating creatinin with barium hydrate, while HUPPERT ob-

tained it by fusing together sarcosin with urea. As it occurs in muscle, it is probably derived from the creatin, though under what conditions this splitting up takes place is not definitely known. Acetic and lactic acids are incapable of effecting this change. At all events, it belongs to the ureids, and is intermediate between creatinin, sarcosin, and urea. Compare the above formula with that of creatinin, p. 349.

Methyl-hydantoïn forms prisms which are easily soluble in water and alcohol, and but slightly soluble in cold ether. It melts at 156° (SALKOWSKI); at 159° – 160° (GUARESCHI and MOSO). Its aqueous solution is slightly acid in reaction. On strong heating it volatilizes. When fused with potassium hydrate it gives off ammonia; it reduces mercuric nitrate in the cold. Treated with mercuric oxid it assumes an alkaline reaction, and the filtrate on heating yields metallic mercury. With silver oxid it forms pearly lanceolate plates having the composition $C_4H_5N_2O_2 \cdot Ag$. It does not give any alkaloidal reactions.

UNDETERMINED LEUCOMAINS.

Leucomains of Expired Air.

It was shown at quite an early period that exhalations from animals contain, besides an increased amount of carbonic acid, some organic matter, the nature of which, on account of the exceedingly minute quantity in which it occurs, has never been satisfactorily determined. RANSOME, in 1870, estimated the organic matter in expired air by permanganate of potash to be about 0.2 g. per day. Later UFFELMANN showed that the amount of the organic matter in occupied closed rooms increased in almost the same ratio as carbonic acid. HERRMANN, however, denied the existence of organic substances in the expired air. Nevertheless, various observers did not hesitate to ascribe to it the ill effects consequent upon breathing impure air, while at the same time the carbonic acid formed during respiration was considered as either entirely

inert or as insignificant in its action. Thus, respired air from which moisture and carbonic acid have been removed, but which still contains the organic vapors, has been found to be highly poisonous. On the other hand, if the respired air is drawn through a red-hot tube to destroy the organic matter, the air thus purified is capable of sustaining life even in presence of a large percentage of carbonic acid. While it cannot be, therefore, doubted that the organic matter of expired air plays a most important part in producing the well-known noxious effects resulting from breathing confined and vitiated air, nevertheless it would seem from experiments made by ANGUS SMITH that the increase of even such small quantities of carbonic acid in the air as from 0.04, the normal amount present, to 0.1 per cent., is capable of producing systemic disturbances characterized by a decrease in the pulse-rate and an increase in the rate of respiration.

SMITH is consequently of the opinion that the constant lowering of the pulse in impure air, occasioned by the presence of carbonic acid, must have a depressing effect on the vitality. Whatever ill effects the carbonic acid may produce of itself, it remained quite certain that this gas was not the most potent and most injurious constituent of respired air; and the investigations of HAMMOND, NOWAK, SEEGEN, and others pointed to the organic matter as the direct and immediate agent which produces those symptoms of sickness and nausea experienced in badly ventilated closed rooms.

Of special importance to the sanitarian and physician is the work on the nature and action of the poisonous principle of expired air which has been done by BROWN-SÉQUARD, D'ARSONVAL, and R. WURTZ. The first two observers found that the vapors exhaled by dogs, when condensed, and the aqueous liquid (20-44 c.c.) thus obtained was injected into other animals, death was produced, generally within twenty-four hours. The symptoms observed were dilatation of the pupil, increase of heart-beat to 240-280 per minute, which may last for several days or even weeks, while the temperature remains normal; the respiratory movements are generally slowed, and

usually there is observed paralysis of the posterior members. Choleraic diarrhœa is invariably present. As a rule, it appears that larger doses cause labored respiration, violent retching, and contraction of the pupil. A rapid lowering of temperature, 0.5° to 5° , is sometimes observed. These same symptoms, apparently in aggravated form, were obtained when the liquid had been previously boiled for the purpose of destroying any germs that might be present. The appearances presented on post-mortem were much like those observable in cardiac syncope.

The above work has been confirmed in part by R. WURTZ, who, by passing expired air through a solution of oxalic acid, has obtained, besides ammonia, a volatile organic base which is precipitated by Bouehardat's reagent and by potassio-mercure iodid. It is said to form a platinum double salt crystallizing in short needles, and a soluble gold salt. When heated to 100° it gives off a peculiar odor. This basic substance may properly be looked upon as a leucomain. The possibility of its being an ammonium compound is not excluded. The existence of a basic product in the expired air has not been confirmed by LEHMANN and JESSEN or by BEN. MERKEL obtained a very small amount of a salt insufficient for study.

DASTRE and LOYE, and LEHMANN and JESSEN and MERKEL have repeated the above experiments with wholly negative results. It is possible that the most highly poisonous substances formed in the body when there is an insufficient air-supply are not eliminated in the exhaled air. Similar negative results were obtained by HOFFMANN-WELLENDORF and by RUSSO-GILBERTI and ALESSI, who injected the condensed moisture from expired air without effect. BEN, in 1893, has again taken up the subject of the toxicity of expired air. From about 3000 liters of his expired air (eight hours) he obtained about 100 c.c. of condensed water having a peculiar, not unpleasant odor. It gives a distinct reaction for ammonia with Nessler's reagent, but contained no alkaloids. The organic substance amounted to 5 mg., or for twenty-four hours

to 15 mg. By repeating WURTZ's experiment with 500 and 700 liters of expired air no alkaloidal reactions were obtained or effects produced in animals. From these and other experiments, he concludes that the organic matter of expired air cannot induce acute intoxication. The dyspnoea observed in confined spaces is due to the lack of oxygen. Carbonic acid may give rise to dulness and headache, but the amount may rise considerably and be harmless so long as oxygen is not decreased too much.

Sewer-air, according to observations made by ODLING, contains a basic substance which is probably in composition a compound ammonia. It contains, however, more carbon than methylamin and less than ethylamin.

It should be remarked that JACKSON (1887) announced the presence in expired air of quantities of carbon monoxid gas sufficient to produce the ill effects ordinarily attributed to the organic matter. The presence of this poisonous gas must first be fully demonstrated before it can be taken into account in the consideration of the toxicity of air; certainly, even if present, it cannot explain the results obtained by the French investigators as stated above.

According to ILOVA, expired air contains nitrous acid. This may possibly be derived from that which is constantly being formed in the mouth, probably by the reduction of nitrates (MILLER).

Leucomäins of the Urine.

A number of basic substances have been isolated at different times from the urine, and on that account they may be properly classed as leucomäins. Thus, LIEBREICH (1869) found in the urine a base which apparently was an oxidation-product of cholin, and which has since been regarded as identical with betain. In 1866 DUPRÉ and BENGE JONES found, among other things in the urine, an alkaloidal body which in sulphuric acid solution possessed a blue fluorescence (see p. 30). Most of the members of the uric acid group of leucomäins

have been detected in the urine and on account of their well-defined nature they are described by themselves.

In 1879, THUDICHUM announced the presence in the urine of four new alkaloids, one of which, urothecobromin, was subsequently rediscovered by SALOMON and named paraxanthin. Another base which was obtained, namely, reducin, yielded a barium salt which readily reduced the salts of silver and mercury. Its formula probably corresponded to $C_{12}H_{24}N_6O_9$ or $C_6H_{11}N_3O_4$. A third alkaloid, parareducin, formed a zinc compound having the composition $C_6H_9N_3O \cdot ZnO$. A fourth base is said to give a compound with platinum chlorid and to contain an aromatic nucleus (aromin). Besides these four bases THUDICHUM describes two other substances which he considered to be basic. These were urochrome, the normal pigment of the urine, and creatinin.

In 1880, POUCHET announced the presence of caruin, $C_7H_8N_4O_3$, and of another base which he subsequently analyzed and found to have either the composition $C_7H_{12}N_4O_2$ or $C_7H_{14}N_4O_2$. This substance formed deliquescent fusiform crystals, sometimes crystallized in bundles or irregular spheres, which possessed a slightly alkaline reaction and combined with acids to form crystallizable salts. It was soluble in dilute alcohol, almost insoluble in strong alcohol, insoluble in ether. The hydrochlorid yielded double salts with gold chlorid, platinum chlorid, and mercuric chlorid. The platinochlorid formed deliquescent golden-yellow rhombic prisms. This base occurred in the dialysate (see page 398). From the non-dialyzable portion, POUCHET obtained another base corresponding to the formula $C_3H_5NO_2$, which he called the "extractive matter of urine." It gave precipitates with the general alkaloidal reagents, was non-crystallizable, and is altered on exposure to air and resinified by hydrochloric acid. On the addition of platinum chlorid it was rapidly oxidized, but did not yield a platinochlorid. The bases were poisonous to frogs, produced paralysis, loss of reflexes, and stoppage of the heart in systole. The same author regarded the urine as containing very small quantities of some pyridin bases, analogous to or

identical with those obtained by GAUTIER and ETARD from decomposing fish.

BAUMSTARK isolated a compound from the urine having the composition $C_3H_8N_2O$. In forty liters of urine it could just be detected; was more abundant in one case of icterus. It was not present in dog's urine, except when it was fed benzoic acid. It crystallized from water in white prisms resembling hippuric acid. The crystals decrepitate on heating; are unchanged at 250° , but at higher temperature give off dense, white vapors having a peculiar odor; melt and take fire. The odor is that of burned horn. It is rather easily soluble in hot water, difficultly in cold water and in alcohol; not in absolute alcohol or ether. The solutions are neutral. It forms easily soluble salts. The hydrochlorid, $C_3H_8N_2O \cdot HCl$, crystallizes difficultly in dendritic masses; is deliquescent and soluble in alcohol. It does not combine with bases, and is precipitated by mercuric nitrate, resembling allantoin and urea. When heated in a glass tube or with soda-lime, it gives off a combustible gas having the odor of ethylamin. On boiling with baryta or ammonia ethylamin and barium carbonate result. With nitrous acid it gives sarcolactic acid. A somewhat similar substance was isolated by MEISSNER from the urine of the dog.

The distinguished Italian toxicologist SELMI was, perhaps, the first to draw attention to the probable formation of basic substances in the living body during those pathological changes brought on by the presence of pathogenic germs; and in a memoir presented to the Academy of Sciences of Bologna, in December, 1880, he announced that infectious diseases, or those in which there occurs an internal disarrangement of some element, either plasmic or histological, must be accompanied or followed by an elimination of more or less characteristic products which would be a sign of the pathological condition of the patient. To support this theory he examined a number of pathological urines, and succeeded in obtaining from them basic substances, some of which were poisonous, others not. Thus, a specimen of urine from a patient with progres-

sive paralysis gave two bases strongly resembling nicotin and coniin; from other pathological urines the bases obtained usually had either an ammoniacal or trimethylamin odor. It is well to note that in normal urine ammonia and trimethylamin are present, while organic bases, as peptotoxin, are absent (STADTHAGEN). SELMI proposed to designate the basic substances found in disease as pathoamins. The term urotoxin is likewise sometimes employed to designate the urine poison. A strong confirmation of SELMI's theory is seen in the observations made by BOUCHARD, VILLIÉRS, LÉPINE, GAUTIER, and others, all of whom have apparently found basic substances in the urine of various diseases.

Thus, BOUCHARD asserted the presence in normal urine of two bases, one soluble in ether, the other insoluble in ether, but soluble in chloroform. By the extraction of urine from typhoid fever, pneumonia, pleuritis, and icterus with ether he obtained substances that gave alkaloidal reactions. LÉPINE and GUÉRIN likewise extracted alkaline urine with ether and obtained a poisonous substance. The extracts from pathological urines were more poisonous than those from normal urine, and the typhoid extract reacted differently from that of pneumonia. VILLIÉRS found the basic substances, as a rule, in pneumonia, tuberculosis, abscesses, but absent in tetanus. In all these cases only extracts were employed, the substance not being isolated in a degree of purity and in amount sufficient for analysis. It is comparatively easy (from the result of the application of the so-called alkaloidal tests) to report upon the presence of alkaloids in so complex a fluid as the urine. It is much more difficult, however, to isolate such bodies in a chemically pure condition, satisfying the requirements of exact research.

The criticisms on these older examinations apply with equal force to many of the more recent investigations of the urine in disease. Thus CHIARUTTINI applied Spica's method for the extraction of ptomaines to the urine of various nervous diseases with convulsions. In twelve cases alkaloids were obtained which produced similar toxic effects in animals. Ars-

LAN from the urine of two children with ankylostomiasis separated a toxin that induced anæmia in rabbits. BOINER and SILBERET isolated three bases from the urine of Basedow's disease that in animals produced similar effects as observed in the disease. MARINO-ZUCO showed that the poisonous action of extract of the adrenals, as observed by FOA and PELLACANI, was due to cholin (neurin of MARINO-ZUCO). With DUTTO, he found in the urine of Addison's disease a base which behaved with reagents like cholin. They therefore consider the disease as due to slow auto-intoxication with this base. It may be mentioned in this connection that eclampsia is considered by FAVRE as a ptomaïnæmia, whereas BOUCHARD regards it as due to the non-elimination of the normal poisons of the urine. There is more reason, however, in considering it as due to perverted cell-metabolism, just as the varnishing of a part or the whole of the skin results, as KLIJANITZIN has pointed out, in the alteration of the chemical products of the underlying cells. The same author has shown the urine, as well as the blood and organs, to contain a basic poisonous substance, presumably peptotoxin (see page 408) in cases with extensive skin-burns.

In 1889 LUFF examined the urine of infectious diseases for basic products by the following method: A large quantity of the urine was rendered alkaline with sodium carbonate, and agitated with one-half its volume of ether. After standing for some time the ether was removed, filtered, and then agitated with a solution of tartaric acid, to remove the alkaloids as soluble tartrates. The aqueous acid solution is then rendered alkaline with sodium carbonate, and agitated with one-half its volume of ether. The ether is removed, allowed to evaporate spontaneously, and the residue, after drying over sulphuric acid, is examined for alkaloids.

The urine of typhoid fever, collected during a high fever for four days, gave a small quantity of a white crystalline substance. When dissolved in hydrochloric acid it gave reactions with phosphomolybdic acid, potassium mercuric iodid, iodine solution, tannic and picric acids, and gold

chlorid; failed to react with phosphotungstic acid and platinum chlorid. The examination of a second case was negative.

Scarlet-fever urine collected during the height of the fever (four gallons) gave a small amount of a white semi-crystalline alkaloid. The solution in water was faintly alkaline. The hydrochloric acid solution did not react with tannic acid or platinum chlorid, but gave precipitates with the other reagents mentioned above. The amount of substance was insufficient to allow of analysis. In normal urines no such residues were found.

HUNTER, in 1890, examined the urine of pernicious anemia by the benzoyl-chlorid method, and obtained a very small quantity of a benzoyl compound, which was extremely soluble in alcohol, insoluble in water. It crystallized from alcohol in long, fine needles, grouped in rosettes. The melting-point was at 174° – 175° . The crystalline form and the melting-point agreed with putrescin. This compound was usually alone, but sometimes was accompanied by another, forming elongated, rectangular prisms. The crystalline form resembled that of the cadaverin compound. One specimen of urine furnished a dibenzoyl compound, crystallizing in long, rectangular prisms having a melting-point between 70° and 80° . BINET isolated a thermogenic substance from the urine of tuberculosis, and to a less extent from normal urine.

A most prolific supply of alkaloids from the urine of infectious diseases has been furnished by GRIFFITHS. During the past four years he has supplied formidable names and formulæ for fifteen bases isolated from the urine of as many infectious diseases. The method employed was identical with that described by LUFF. A list of these bases, together with others, is given on page 320.

Certain basic substances, as the diamins, cadaverin, and putrescin, have been isolated in a perfectly pure condition. These two basic substances (see page 325) were observed by BAUMANN and UDRÁNZSKY in one case of cystinuria. Later,

BRIEGER and STADTHAGEN demonstrated the presence of these same bases in two or more cases of cystinuria. They are absent in normal urine and feces, and exceedingly rare in other diseases. Thus, ROOS has found diamins (putrescin) in only one case of cholera, and then in the feces, not in the urine; more frequently in diarrhoea or cholera; also in one case of dysentery and malaria. As stated above, HUNTER has succeeded in apparently isolating putrescin from the urine of pernicious anæmia.

POEHL has proposed the following method for the estimation of the leucomains in the urine. To 100 c.c. of the urine, 25 c.c. of hydrochloric acid (sp. g. 1.134) and 10 c.c. of a 10 per cent. solution of phosphotungstic acid are added. Albumin and pepton must first be removed if present. The precipitate is allowed to subside in a graduated tube, and the number of cubic centimetres occupied by the precipitate divided by 8 is to represent the approximate weight of leucomains per liter of urine. The amount thus found in the two cases was 0.6 and 1.69. CAVALLERO and OLIVETTI have, with justice, severely attacked this method, and have shown its utter unreliableness.

It is now a well-established fact that the urine of certain infectious diseases, as cholera (BOUCHARD) and septicæmia (FELTZ), etc., is far more poisonous than normal urine. That the poisons, basic or otherwise, which are generated within the body by the activity of bacteria can be excreted in the urine is seen in the fact that immunity to the action of bacillus pyocyaneus has been conferred on animals by previous injection of urine taken from animals inoculated with that bacillus (BOUCHARD) or with filtered cultures of the same (CHARRIN and RUFFER).

Furthermore, the excretion of the tetanus and diphtheria poisons by the urine has been shown to take place. Thus, BRUNNER demonstrated the tetanus poison in the urine of experimental animals, but failed with the urine of the disease in man. BRUSCHIETTINI, however, with the urine of a tetanus

patient, produced tetanic symptoms in mice by the injection of 3-10 c.c. subcutaneously. In the urine from diphtheria patients ROUX and YERSIN demonstrated the presence of the diphtheritic poison by inducing paralysis in animals. Although basic substances are not present in the urine of cholera, they are present, but less frequently than was expected, in the discharges (putrescin in only one of four cases, ROOS). From cholera-feces POUCHET extracted an oily fluid very poisonous to frogs; whereas, VILLIERS obtained a base which produced convulsions in guinea-pigs. KULNEFF pointed out the presence of ethylenediamin (?) in the stomach-fluids of gastrectasia, while from the feces of a case of gastroptosis he isolated trimethylamin.

In the consideration of the toxins in the urine of infectious diseases it must not be forgotten, as pointed out by JAWELN, that the poison as well as the specific germ may be present in the urine. Thus, in rabbits that died as a result of infection with anthrax-bacillus, erysipelas-streptococcus, Eberth's bacillus, and Fraenkel's diplococcus, the urine was found to contain these organisms. It, therefore, becomes difficult to decide as to whether the toxin is elaborated within the body or formed subsequently to the secretion of the urine.

The question of the toxicity of normal urine has been the subject of considerable controversy. The early explanations of the cause of uræmia assumed that urine was poisonous, and that uræmic symptoms were the result of the retention of urine. Actual demonstrations of the toxicity of urine were made early in the century by VAUQUELIN and others. On the other hand, disbelievers in the toxicity of urine were not wanting. Thus FRERICUS maintained that death, resulting from intravenous injections of urine, was due to suspended solid elements of the urine; that urea itself was harmless, but that it could by the action of a ferment give rise to the poisonous ammonium carbonate. VOIT was among the first to point out that potassium salts, on account of their toxicity, could play an important part in uræmia. It can now be positively stated that normal urine does possess a certain

degree of toxicity. It is more difficult to decide upon the nature of this poison. FELTZ and RITTER (1881), and independently ASTASCHIEWSKY, arrived at the opinion that the toxicity was chiefly due to the potassium salts of the urine. SCHIFFER, while acknowledging the presence and action of the inorganic salts, maintained that the urine contained a definite organic poison, for the reason that the concentrated aqueous solutions from alcoholic extracts of the urine-residue killed large rabbits in doses corresponding to 1-1½ liters of urine, deprived of inorganic salts.

According to BOUCHARD, 30 to 60 c.c. of normal urine, injected intravenously, will kill a rabbit weighing one kilogram. Hence a man weighing 60 kilograms, and excreting per day 1200 c.c., would, if 50 c.c. are necessary to kill one kilogram of living matter, secrete enough poison to kill twenty-four kilograms of animal. Inasmuch as the amount necessary to kill one kilogram of animal is designated as one urotoxy, therefore, in the above case twenty-four urotoxies are formed per day. The urotoxic coefficient is the number of urotoxies which one kilogram of man forms in twenty-four hours. Therefore, $\frac{24}{60}=0.4$, the urotoxic coefficient. The average normal urotoxic coefficient is placed by BOUCHARD at 0.464. It follows, therefore, that an average man would, if the excretion of urine was stopped, be killed in fifty-two hours. The variations of the urotoxic coefficient in the normal individual is limited. In disease it rarely exceeds 2, and rarely falls below 0.10.

According to BOUCHARD, five kinds of poisons may be met with in the urine, producing narcosis, salivation, mydriasis, paralysis, and convulsions. The day urine, which is chiefly narcotic, is 2-4 times more toxic than the sleep urine, which induces convulsions and is antagonistic of the former. The toxicity is independent of the density, since night urine is more dense than that secreted during the day.

The greater part of the toxicity of urine is ascribed by BOUCHARD to organic poisons, especially coloring-matters, whereas potassium salts are regarded as the cause of but a small fraction of the toxicity.

LÉPINE likewise found that about 60 c.c. of urine sufficed to kill one kg. of animal. The inorganic salts, however, are ascribed by him a much greater importance, inasmuch as he estimates that 85 per cent. of the intoxication is due to this cause. The remainder of the toxicity is due to organic matter. STADTHAGEN has arrived at practically the same results, that 80–85 per cent. of the toxicity is due to the inorganic constituents. A part of the toxicity, 15–20 per cent., is therefore due to organic substances. No one organic substance in the urine, as urea, creatin, etc., possesses this toxicity. STADTHAGEN has further shown that alkaloidal bodies as peptotoxin, guanidin, methyl-guanidin, cholin, neurin, xantho-creatinin are absent from normal urine. 100 liters of urine examined by Brieger's method for bases gave only ammonia, a little trimethylamin, besides creatinin. GAUTIER has supposed that the urine poison was a proteid analogous to that in the venom of serpents, but STADTHAGEN showed that proteids were absent.

Ferments like pepsin are also excluded from consideration because of their minute amount, while his experiments were largely negative, so far as the detection of an organic poison was concerned. STADTHAGEN disproved the existence of a special urine poison. The poisonous action of normal urine is therefore due to the sum total action of the inorganic salts, chiefly potassium, and the normal organic constituents as urea, creatinin, etc., which by themselves possess but a slight action.

GUINARD has recently tested the action of normal urine from different animals. On an average, the toxicity per kg. rabbit was as follows: Dog, 193 c.c.; man, 132.7 c.c.; pig, 53 c.c.; ox, 38.5 c.c.; guinea-pig, 35 c.c.; sheep, 33.8 c.c.; goat, 32 c.c.; ass, 29.4 c.c.; horse, 29.2 c.c.; rabbit, 16 c.c.; cat, 13 c.c. The urine of a bear possessed toxicity similar to that of the dog; that of the lion and tiger corresponded to that of the cat. In the case of the horse the urine was less toxic from weak animals, from young animals, and from males than from strong or old animals or females. The urea

per liter of urine varied from 15 g. in the dog to 118 g. in the cat. While rabbits are killed, per kilo, by an injection of 45 c.c. of normal urine, dogs are killed by an intravenous injection of 100 c.c. per kilo (MAIRET and BOSC). If the thyroid gland is removed, the toxic effects are increased (GODART and SLOSSE).

While GUINARD failed to observe any effect or toxicity of urine by pregnancy, CHAMBRELENT and DEMONT found that it was diminished in the later months. The average urotoxic coefficient was 0.27. MAIRET and BOSC examined the toxicity of the urine in nervous disorders and found it to be increased, especially in lypemania and mania. In general, however, the toxic action was the same as that of normal urine, though at times it produced specific nervous symptoms approximating those of the disease.

Increased toxicity of the urine was observed by SURMONT in atrophic cirrhosis, tuberculosis, and carcinoma of the liver. On the other hand, the toxicity was normal or subnormal in hypertrophic cirrhosis, in hepatic congestion, and in infectious icterus.

ROQUE and LEMOINE showed that there are marked changes in the toxicity of the urine in malaria before and after an attack. Before an attack the urotoxic coefficient was 0.13 and 0.274, whereas after an attack it rose to 0.684 and 1.276. It would appear, therefore, that toxic products result from the growth of the malaria plasmodium in the blood, and are largely eliminated by the kidneys. Quinin favors this excretion of poisons.

It does not follow from what has been stated that the urine of disease is always more poisonous than in health. There are diseases, as uræmia, where, as shown by SCHIFFER and by BOUCHARD, the urine is less toxic than in health. In this disease or condition this may be due to a retention of the salts of potassium.

Leucomains of the Saliva.

According to GAUTIER (1881), normal human saliva contains divers toxic substances in small quantities which differ

very much in their action according to the time of their secretion, and probably according to the individual gland in which they are secreted. The aqueous extract of saliva at 100° is poisonous or narcotic in its action toward birds. To show the presence of basic substances, the aqueous extract was slightly acidulated with dilute hydrochloric acid, then precipitated by Mayer's reagent; the precipitate was washed, then decomposed by hydrogen sulphid, and the solution filtered. The filtrate on evaporation gave a residue consisting of microscopic slender needles of a soluble hydrochlorid. This salt, purified by extraction with absolute alcohol, formed soluble, crystalline, but easily decomposable double salts with platinum chlorid and with gold chlorid. The solution of the hydrochlorid produces an immediate precipitate of Prussian blue in a mixture of potassium ferrieyanid and ferric chlorid, and when injected into birds produces stupor.

Leucomains from other Tissues of the Body.

SELMER'S work upon the formation of ptomaines during the process of putrefaction led many investigators to doubt the production of these bases by the decomposition of the proteid or other complex molecules. To substantiate this, a number of chemists, especially Italian, endeavored to show that SELMER'S bases, to a large extent at least, exist preformed in the various tissues. PATERNO and SPICA (1882) succeeded in extracting from fresh blood as well as from fresh albumin of eggs substances identical, or at least similar, to those designated under the name of ptomaines. Their observations, however, were confined to the detection of alkaloidal reactions in the various extracts obtained by Dragendorff's method, and at no time were they in possession of a definite chemical individual. MARINO-ZUCO (1885) was more successful, inasmuch as he succeeded in obtaining from fresh tissues and organs relevant quantities of a base identical with cholin, and, in addition, he obtained extremely minute traces of other alkaloidal bodies. One of these, obtained by the Stas method

from the liver and spleen of an ox, exhibited in hydrochloric acid solution a beautiful violet fluorescence resembling very much that of the salts of quinin. A similar base, probably identical with this one, was obtained by BENCE JONES and DUPRÉ (1856) from liver, nerves, tissues, and other organs, and was named by them "animal chinoidin." A greenish-blue fluorescence is frequently observable in the alcoholic extracts of decomposing glue as well as from other putrefying substances, and is undoubtedly due to products formed by some one of the fluorescing bacteria. From a number of very thorough experiments, he concluded that basic substances do not pre-exist in fresh organs, but that the acids employed in the process of extraction exert a decomposing action upon the lecithin present in the tissues, resulting in the formation of cholin. He further showed that the method of Dragendorff, on account of the larger quantity of extractives which forms, invariably gave a larger yield of this base than did the Stas-Otto method. Similar observations were made by GUARESCHI and MOSO, by COPPOLA and others. At the present time there is no doubt that some basic substances, among these cholin, can be formed by the action of reagents, and, on the other hand, it is equally well demonstrated that similar bases do pre-exist in the physiological condition of the tissues and fluids of the body.

Recently R. WURTZ has obtained from normal blood a number of crystalline products of alkaline reaction, which form well-crystallizable double salts with gold, platinum, and mercuric chlorids. These, however, have not been as yet subjected to analysis, because of the minute quantities which were isolated.

MARINO-ZUCO and MARTIN in 1894 showed the presence of cholin in fresh blood.

In extensive skin-burns KIJANITZIN isolated a peptotoxin-like base from the urine and blood, more abundantly from the organs. A similar base was shown by him to be produced by the action of gastric juice on the blood in the presence of bacteria; also in the early stages of the decomposition of

blood. The explanation of the fatal results following the varnishing of a part or the whole of the body is given on page 409. A similar explanation undoubtedly holds true for uræmia.

The presence of specific toxic substances in the blood of infectious diseases is well recognized. NISSEN has shown that the blood in suppuration was toxic. In the blood of tetanus in man KALLMEYER and NISSEN demonstrated the presence of the tetanic poison. IMMERWAHR showed the same to be true with the organs of experimental tetanic animals, and that the blood of scarlet fever during uræmia was toxic. BRIEGER was the first to show the presence of tetanin in the amputated arm of a patient.

MORELLE (1886) showed the presence, in the spleen of the ox, of a base, the hydrochlorid of which crystallized in deliquescent needles and likewise formed crystalline platino- and aurochlorids. From experiments made by LABORDE, the base would seem to possess decided toxic properties, bringing on a dyspnoic condition with convulsive movements and loss of motion. The post-mortem examinations revealed an extended visceral œdematous infiltration, and stoppage of the heart in systole. For the presence of the xanthin bases, cystin, gerontin, etc., in the organs of the body, see preceding pages.

VIRON found a very poisonous albuminoid in a hydrocele fluid from a sheep.

A. W. BLYTH has claimed to have isolated from milk two alkaloidal substances, namely, galactin, the lead salt of which is said to have the formula $Pb_2O_3 \cdot C_{54}H_{18}N_4O_{25}$, and lactochrome, the mercury salt of which is represented by the formula $HgO \cdot C_6H_{18}NO_6$.

Venoms of Poisonous Serpents.

The study of the chemistry of the venoms of serpents and of batrachians is fraught with so many difficulties and with so much danger, that we cannot wonder at the present unsat-

isfactory condition of our knowledge in regard to the poisonous principles which they contain. Much of the work that has been done hitherto is not only inaccurate and very contradictory, but is far from meeting the requirements of exact toxicological research. From recent investigations it seems, however, to be quite certain that the most active constituent of the venom of serpents is not alkaloidal in its nature, as has been supposed by some. In 1881 GAUTIER announced the isolation of two alkaloids from the venom of the cobra which gave precipitates with tannin, Mayer's reagent, Nessler's reagent, iodine in potassium iodide, etc. They formed crystallizable platinochlorides and aurochlorides, and also crystalline, neutral, somewhat deliquescent hydrochlorides. The neutral or slightly acid solutions produced an immediate precipitate of Prussian blue in a mixture of potassium ferri-cyanide and ferric chloride. The substances possessed a decided physiological action, but GAUTIER himself did not consider them to be the most dangerous constituents of the venom. This observation of GAUTIER as to the presence of distinct basic substances in venoms is at variance with that of WOLCOTT GIBBS, who was unable to obtain an alkaloid from the rattlesnake (*Crotalus*) venom. S. WEIR MITCHELL and E. T. REICHERT were likewise unable to substantiate GAUTIER's statements. Still more recently WOLFENDEN, in an elaborate paper on the nature of cobra-venom, has confirmed WOLCOTT GIBBS as to the entire absence of any alkaloidal body.

MITCHELL and REICHERT made a careful study of the venoms of various serpents, such as cobra, rattlesnake, moccasin, and Indian viper, and succeeded in isolating two proteid constituents, one belonging to the class of globulins and the other to the peptons. The pepton is said to be non-precipitable by alcohol. According to them, the globulin constituent consisted of at least three distinct globulins. They found that boiling coagulated and destroyed the globulin as a poison, but that the venom pepton was toxically unchanged, so that the solution, though still poisonous, fails to produce the characteristic local lesions due to fresh or

unboiled venom. On the other hand, GAUTIER asserted that the venom was not sensibly altered on being heated to 120° – 125° , and that the toxic action remained constant even when all the proteid constituents are removed thus showing that the toxic action cannot be attributed to the albuminoids. Later, QUARTIER acknowledged that viper venom was destroyed at 100° . CALMETTE found it to be destroyed at 98° , while still later PHISALIX and BERTRAND showed that an exposure of five minutes at 80° – 85° destroyed the toxicity. The venom pepton from the rattlesnake or the moccasin, however, when injected into animals produced toxic effects which were marked by an œdematous swelling over the site of injection; the tumor was filled with serum, and so also was the subcutaneous cellular tissue. Furthermore, a gradual breaking down of the tissues occurred, accompanied by rapid putrefactive changes and a more or less extensive slough. That peptons may possess intensely poisonous properties has been shown to be the case by a number of authors, among whom may be mentioned SCHMIDT-MÜLHEIM, HOFMEISTER, POLLITZER, and others. BRIEGER has, moreover, demonstrated that the formation of peptons in the process of digestion is accompanied by the development of a toxic ptomain, which he has named peptotoxin. As stated elsewhere, SAL-KOWSKI doubts the formation of peptotoxin in ordinary digestion of proteids.

The venom globulins, on the other hand, though present in less quantity than the peptons, induced the same remarkable local effects seen on injection of the pure venom. They caused local bleedings, destroyed the coagulability of the blood, and rapidly corroded the capillaries.

These results of MITCHELL and REICHERT, which are given here somewhat in full, have been questioned by WOLFENDEN, who, while agreeing in the main that the poisonous property of venom is due to proteid constituents, regarded their pepton not as a true pepton, but rather as one or more bodies of the albumose group of proteids. He likewise regards the globulin of moccasin venom to be some other proteid body.

According to him, the cobra venom owed its toxicity to the proteids, globulin, serum albumin, and acid albumin. Occasionally there seem to be present traces of pepton and of hemialbumose.

BRIEGER was at first apparently inclined to believe that the action of venom is due to animal alkaloids, on the ground that these bases are extremely soluble, and hence always go into solution, along with the likewise very soluble proteid constituents, and that the difficulty in their isolation lies in the elimination of these proteids. Since then BRIEGER and FRAENKEL pointed out the poisonous nature of some bacterial proteids, and also showed that cobra poison yields with alcohol a precipitate which gives proteid reactions.

The proteids of serpents' venom should be compared with the poisonous proteids formed by the activity of the pathogenic bacteria, and more especially with the bacterial toxins, also with similar compounds, the *phytalbumoses* of castor seeds, jequirity, etc., and with the enzymes. Possibly similar compounds will be found in croton and other species of ricinus, jatropha, loco-weed, etc. The poisons secreted by certain spiders and fish may be mentioned in this connection.

The researches of the past two years on the venom of serpents have been productive of very important results, especially from the standpoint of immunity to and cure from the bites of venomous serpents. Although in the higher latitudes poisoning from snake-bites is comparatively rare, it should not be overlooked that in certain portions of the globe, notably India and Australia, the mortality from this cause is exceedingly high, and may well claim the attention of governments. Thus, it is estimated in India that over 20,000 persons die annually from the bites of serpents.

PHISALIX and BERTRAND in 1893 confirmed FONTANA's previous observation that the garter-snake (conleuvre) was unaffected by repeated bites from vipers or by subcutaneous injection of the venom of the viper. These authors showed that a dose of the venom sufficient to kill 15-20 guinea-pigs was without effect on the garter-snake. The natural immu-

nity of the garter-snake to the viper venom is thus firmly established. From previous researches on the natural immunity of the "crepaud" and viper to their own venoms PHISALIX and BERTRAND showed that the blood or serum of these serpents contained the same poison, *echidnin*, as was present in the venom. Similarly the blood or serum of the garter-snake when injected in doses of 1.5 c.c. intraperitoneally into guinea-pigs produced death in two hours with the same symptoms as are observed after poisoning with viper venom. Although the several forms of garter-snakes are considered as non-venomous, they nevertheless secrete through the superior maxillary gland (or special glands, JOURDAIN) toxic products analogous to echidnin, which are excreted into the blood, rendering this, therefore, highly poisonous, and at the same time establishing natural immunity. After the ablation of the venom glands in the viper the blood loses a part of its toxicity, showing that the source of the poison in the blood is the venom gland. It would seem that this immunity is one of tolerance and analogous to that which SEWALL obtained with rattlesnake venom.

Later (February, 1894), PHISALIX and BERTRAND showed that viper venom heated to 75° – 85° for five minutes lost its poisonous property with respect to guinea-pigs and acted as a vaccine. The temperature of animals, however, was raised, whereas with unheated venom it is lowered. They were, therefore, led to believe that viper venom contained (1) a phlogogenic substance like the diastases—echidnase, and (2) a general poison—echidnotoxin. Since both are destroyed by heat the vaccine results either from the destruction of these two substances or is preformed in the venom and acts after the toxic principles are destroyed. This behavior of venom to heat and to the production of immunity is analogous to Fränkel's method of producing immunity to diphtheria.

The heated viper venom, or vaccine, does not impart immediate immunity to guinea-pigs, but this condition follows after the lapse of several days—a result of the reaction of the organism. An antitoxin appeared in the blood after the injec-

tion of the echidnovaccine, and in less amount in the blood when immunity has been established by tolerance. The amount of the antitoxin in the blood could be increased as in the case of tetanus and of diphtheria. A very short time afterward CALMETTE confirmed the observations that animals could be immunized by repeated injections of the venom, beginning in small doses and gradually increasing. A single non-fatal injection of venom may produce antitoxin in the blood of the animal. He furthermore obtained immunity by applying the method employed by ROUX and VAILLARD in their work on tetanus, that is, by repeated injections of the venom mixed with gold chlorid, or sodium or calcium hypochlorite. The serum of the immunized animal was found to be antitoxic in the same sense as the serum of animals immunized to diphtheria or tetanus. Furthermore, not only was the blood shown to be antitoxic to the venom employed, but also to the venoms of other serpents. Thus, the serum of a rabbit immunized against the cobra venom is not only antitoxic to this venom, but also to the viper of France, the black snake of Australia, etc.

Immunity therefore to venom can be obtained (1) by repeated injections of small doses (SEWALL, PHISALIX and BERTRAND, CALMETTE); (2) by the use of heated venom or vaccine (PHISALIX and BERTRAND); (3) by repeated injections of venom mixed with hypochlorite solution (CALMETTE); (4) by injections of antitoxic serum (PHISALIX and BERTRAND, CALMETTE). The immunity according to the first method, by tolerance, has been shown to be due to the presence of antitoxin substances in the blood (PHISALIX and BERTRAND, CALMETTE, FRASER). The second and third methods are explainable in the same way.

The application of the latter principle in the treatment of bites from serpents was suggested by PHISALIX and BERTRAND and carried out by CALMETTE. The rabbit, dog (?), guinea-pigs (PHISALIX and BERTRAND), horse and ass have been employed to furnish serum antitoxic to venom. CALMETTE has prepared a serum of a strength of 1:10,000; that is,

rabbits given a dose of venom sufficient to kill in three or four hours are saved if a quantity of antitoxic serum corresponding to $\frac{1}{100000}$ of their weight is injected not later than one hour after the injection of venom. As stated above, this antitoxic serum protects against all venoms.

CALMETTE has also shown that the ichneumon of the Antilles is naturally immune to venom, and that it owes this condition to the antitoxic property of its blood. Just as the serum of man was sometimes found to be antitoxic to the diphtheria poison, so the serum of dogs was occasionally found to be antitoxic to venom.

FRASER has independently arrived at substantially the same results as the French investigators. Serum that is antitoxic to venom is designated by FRASER as antivenen. He immunized the horse and cat against the cobra venom. The cat was also rendered immune by administration through the stomach. It is interesting to note that RÉPIN obtained immunity in guinea-pigs to abrin, the poisonous allumose of jequirity, by repeated administrations of small doses by the mouth. EHRLICH has shown that while $\frac{1}{10}$ mg. of abrin is sufficient to kill a guinea pig in two or three days when injected subcutaneously, one hundred times this amount, 10 mg., is necessary to kill by the mouth. ROUX and YERSIN endeavored to produce immunity to diphtheria by the mouth, but were unsuccessful.

According to FRASER, 0.18 mg. of cobra venom constitutes the minimum fatal dose for 1 kilogram of rabbit. The guinea-pig is less susceptible, and the kitten still less so, requiring 2 mg. The minimum fatal dose of the rattlesnake venom, per kilogram of rabbit, is placed at 4 mg. The cobra venom is, therefore, 16–20 times more powerful than that of the rattlesnake.

CALMETTE has successfully saved rabbits from intoxication by venom by injecting in a circle, at a distance from the wound, a solution of fresh calcium hypochlorite. In the case of man an injection of 20–30 c.c. of the fresh solution obtained by diluting a 1:12 solution (5 c.c.) with boiled

water (45 c.c.). MAIRET and BOSC consider this protection by hypochlorite solution as due to a direct action on the venom poison, and not to the formation of antitoxin. This method of treating venom-bites has been tried with success in Australia. It should be noted that a solution of hypochlorite not only destroys the poison of venom, but also the toxin of glanders (PENCII), of tetanus, and diphtheria (ROUX).

It is therefore evident that a striking similarity exists in the action of venom of plant albumoses, of bacterial toxins, and of enzymes. The similarity is strengthened further by the behavior of these poisons to heat, to chemicals, and lastly by development of antitoxic substances in the blood of animals artificially immunized against these poisons.

The blood or serum of the common turtle(?) (*Bufo vulgaris*) is in 1 c.c. dose toxic to frogs. This property of the blood, therefore, is a result, as in the case of the viper and the garter-snake, of the "inner secretion" of toxic glands (PHISALIX and BERTRAND).

CLOEZ and GRATIOLET in 1852 examined the poison contained in the cutaneous pustules of some batrachians, and succeeded in extracting a substance which gave a white precipitate with mercuric chlorid and formed a platinum double salt. Beyond this meagre information very little is known in regard to the character of these poisons, though ZALESKY, in 1866, announced the isolation of an alkaloid to which he assigned the formula $C_{34}H_{60}N_2O_5$, and which he named salamandarin. According to DUTARTRE (1890), this base is a leucomain, and similar products, but with different physiological action, are to be found in other batrachians, as the toad, triton(?), green and red frogs, and in the epidermis of some fish. According to CALMEIL, the poison from the toad contains methyl-carbylamin and isocyanacetic acid. According to PHISALIX and CONTEJEAN, the blood of the salamander possesses antitoxic action with reference to curare. The salamander, therefore, is naturally immune, and, moreover, its blood will protect frogs against curare.

TABLE OF LEUCOMAINS.

Formula.	Name.	Discoverer.	Source.	Physiological action.
$C_5 H_6 N_5$	Adenin.	Kossel.	Nuclein-containing organs.	Non-poisonous ; muscle-stimulant.
$C_5 H_4 N_4 O$	Hypoxanthin.	Scherer.	Nuclein-containing organs.	Non-poisonous ; muscle-stimulant.
$C_6 H_5 N_5 O$	Guanin.	Unger.	Nuclein-containing organs, guano.	Non-poisonous ; muscle-stimulant.
$C_5 H_4 N_4 O_2$	Xanthin.	Marcet.	Nuclein-containing organs, calcuil.	Non-poisonous ; muscle-stimulant.
$C_6 H_6 N_4 O_2$	Heteroxanthin.	Salomon.	Urine.	Poisonous.
$C_6 H_6 N_4 O_2$	Methyl-xanthin.	Bondzynski and Gottlieb.	"	"
$C_7 H_8 N_4 O_3$	Paraxanthin.	Thudichum.	"	"
$C_7 H_8 N_4 O_3$	Carnin.	Salomon.	"	"
$C_4 H_6 N_3 O(?)$	Episarkin.	Weidel.	Liebig's meat-extract.	Non-poisonous ; muscle-stimulant.
$C_{10} H_{13} N_9 O_2$	Epignauin.	Balke.	Urine.	"
		Krüdger and Wulff.	"	"
$C_{21} H_{30} N_{16} O_4$	Cytosin.	Kossel and Neumann.	Adenylic acid.	
$C_4 H_5 N_5 O$	Pseudoxanthin.	Gautier.	Muscle.	
$C_3 H_8 N_2 O$	Unnamed.	Baumstark.	Urine.	
$C_5 H_{14} N_2$	Gerontin.	Grandis.	Liver of dogs.	Poisonous.
$C_6 H_{14} N_2$	Spermin.	Schreiner.	Sperma, in tissues of leucocythæmies.	Non-poisonous.
	Protamin.	Miescher.	Salmon-spawn.	
$C_5 H_8 N_4 O$	Cruso-creatinin.	Gantier.	Muscle.	
$C_6 H_{10} N_4 O$	Xantho-creatinin.	"	"	Poisonous.
$C_9 H_{10} N_7 O_4$	Amphi-creatin.	"	"	
$C_{11} H_{24} N_{10} O_5$	Unnamed.	"	"	
$C_{12} H_{26} N_{11} O_6$	"	"	"	
$C_7 H_{12} N_4 O_2$	"	Pouchet.	Urine.	
$C_3 H_5 NO_2$	"	"	"	
$C_{34} H_{60} N_2 O_5$	Salamandarin.	Zalesky.	Salamander.	Poisonous.

CHAPTER XIV.

THE AUTOGENOUS DISEASES.

ALL living things are composed of cells. The simplest forms of life are unicellular, and in these all the functions of life devolve upon the single cell. Absorption, secretion, and excretion must be carried on by the same cell. A collection of unicellular organisms might be compared to a community of men with every individual his own tailor, shoemaker, carpenter, cook, farmer, gardener, blacksmith, etc. However, only the lowest forms of life are unicellular; all others are multicellular. In the higher animals there is a differentiation not only in the size and structure of the cells, but in the labor which they perform. The body of man may be compared to a community in which labor has been specialized. Certain groups of cells, which we designate by the term "organ," take upon themselves the task of doing some special line of work, the well-doing of which is essential to the health, not only of that group, but of other groups as well, or of the body as a whole. There is an interdependence among the various organs. Certain groups of cells supply the fluids or juices which act as digestants, and among these there is again a division of labor. The salivary glands supply a fluid which partially digests the starch of our food: the peptic glands supply the gastric juice which does the preliminary work in the digestion of the proteids; while the pancreatic juice completes the digestion of the starches begun in the mouth, of the proteids begun in the stomach, and does the special work of emulsifying the fats. But even some of these products of complete digestion would be harmful should they enter the circulation unchanged. The peptons must be converted into serum albumin by the absorbing mechanism of the walls of the

intestines, and while 10 per cent. of the fat of the food is split up into glycerin and fatty acids by the action of the pancreatic juice, a much smaller per cent. enters the thoracic duct in this divided form. The food may be taken in proper quality and quantity; the digestive juices may do their work promptly and properly, but if the absorbents fail to perform their functions properly disease results. It may happen that the failure lies in improper or imperfect assimilation, and the result becomes equally disastrous, and with the effects of non-elimination we are fairly conversant. Of the myriads of cells in the healthy human body there is none which is superfluous. It is true that among these ultimate entities of existence death is constantly occurring, but in health regeneration goes on with equal rapidity, and each organ continues to do its daily and hourly task. The microscope has made us familiar with the size and shape of the various cells of the body, and students of pathology have described the alterations in form and size characteristic of various disease-states. But we must remember that in the study of these ultimate elements of life there are other things beside their morphological history to investigate. They are endowed with life, and they, as well as the germs, have a physiology and chemistry which we know but slightly. They are influenced beneficially or harmfully, as the case may be, by their environment. They grow and perform their functions properly when supplied with the needed pabulum. They are not immune to poisonous agents. They are injured when the products of their own activity accumulate about them.

The object in writing this chapter has been to collect what evidence we may concerning those diseases which arise from imperfect or improper activity of the cells of the body, not due to the introduction of foreign cells. To designate this class of diseases we have selected the word *autogenous*, and we understand that in these diseases the *materies morbi* is a product of some cell of the body, and not, as in the case of the infectious diseases, of cells introduced from without the body.

It is true, without exception so far as we know, that the excretions of all living things, plants and animals, contain substances which are poisonous to the organisms which excrete them. A man may drink only chemically pure water, eat only that food which is free from all adulterations, and breathe nothing but the purest air, free from all organic matter, both living and dead, and yet that man's excretions would contain poisons. Where do these poisons originate? They are formed within the body. They originate in the metabolic changes by which the complex organic molecule is split up into simpler compounds. We may suppose—indeed, we have good reason for believing—that the proteid molecule has certain lines of cleavage along which it breaks when certain forces are applied, and that the resulting fragments have also lines of cleavage along which they break under certain influences, and so on until the end-products, urea, ammonia, water, and carbon-dioxid, are reached; also that some of these intermediate products are highly poisonous has been abundantly demonstrated. The fact that the hydrocyanic acid molecule is a frequent constituent of the leucomains is one of great significance. We know that chemical composition is an indication of physiological action, and the intensely poisonous character of some of the leucomains conforms to this fact. It matters not whether the proteid molecule be broken up by organized ferments, bacteria, or by the unorganized ferments of the digestive juices, by the cells of the liver, or by those still unknown agencies which induce metabolic changes in all the tissues—in all cases poisons may be formed. These poisons will differ in quality and quantity according to the proteid which is acted upon, and according to the force which acts.

Peptons and albumoses formed during digestion do not in health reach the general circulation. When injected directly into the blood they act as powerful poisons. They destroy the coagulability of the blood, lower blood-pressure, and in large quantities cause speedy death. BRUNTON attributes the lassitude, depression, sense of weight in the limbs, and dulness in the

head occurring in the well-fed, inactive man, after his meals, to poisoning with peptons. The remedy which he proposes is less food, especially less nitrogenous food, and more exercise. That some substance resulting from the proteids of the food is the cause of this trouble BRUNTON thinks is evidenced by the fact that the weakness and languor are apparently less after meals consisting of farinaceous foods only.

That pepton finds its way into the general circulation frequently is shown by its detection in the urine in many diseased conditions, some of which are infectious and others autogenous in character. However, propeptonuria, or albumosuria, is more common than peptonuria, and we have already seen that many of the bacterial albumoses are among the most highly poisonous bodies known, but the action of the albumoses formed during digestion has not, so far as we know, been studied. The valuable work of KÜHNE and CHITTENDEN on the chemical character of these bodies should be supplemented by a thorough investigation of their physiological effects when injected into the blood. It is more than probable that valuable information would be secured by such studies. That albumose is frequently found in the urine is shown by the following list of diseases in which it has been observed, given in the last edition of the work of NEUBAUER and VOGEL on the urine: KÖSNER has found it in spermatorrhœa; KÖPPEN, in mental diseases without spermatorrhœa; KAHLER, in osteomalacia; BENGE JONES, in multiple myeloma; SENATOR and others in dermatitis, intestinal ulcer, liver-abscess, croupous pneumonia, apoplexy, vitium cordis, resectio-coxæ, parametritis, endocarditis, typhoid fever, nephritis, phthisis, etc.; LOEB, in measles and scarlet fever; LEUBE, in urticaria; and LASSAR, after injections of petroleum. KÖTTNITZ, FURSTNER, and others, find albumose frequently in the urine in mental diseases. Evidently, there is much to learn from the study of the conditions accompanied by the elimination of the albumoses in the urine. It is more than probable that the acute Bright's disease following scarlet fever, diphtheria, and the other acute infectious diseases, owes its exist-

ence to the toxins of these diseases. PRIOR has shown that undigested egg-albumin is sometimes absorbed and produces marked disturbances. A boy, after eating sixteen raw eggs, had a high fever accompanied by the appearance of both albumin and hæmoglobin in the urine.

BRIEGER obtained by digesting fibrin with gastric juice a substance which gives reactions with many of the general alkaloidal reagents, and to which he has given the name "peptotoxin." A few drops of a dilute aqueous solution of this substance sufficed to kill frogs within fifteen minutes. The frogs became apparently paralyzed, and did not respond to stimuli. Slight tremor was perceptible in the muscles of the extremities. Rabbits of about one kilogram weight were given from 0.5 to 1 gram of the extract subcutaneously. About fifteen minutes after the injection paralysis, beginning in the posterior extremities, set in; the animal fell into a somnolent condition, sank and died. In some rabbits several hours elapsed before the above mentioned symptoms appeared.

Peptotoxin was found by BRIEGER to be formed not only by the digestive juice, but to be among the first putrefactive products of proteids, as fibrin, casein, brain-substance, liver, and muscle.

It is highly probable that many of the nervous symptoms which accompany some forms of dyspepsia are due to the formation and absorption of poisonous substances.

In some persons the tendency to the formation of poisons out of certain foods is very marked. Thus, there are some to whom the smallest bit of egg is highly poisonous; with others milk will not agree; and instances of this kind are sufficiently numerous to give rise to the adage, "What is one man's meat is another man's poison."

BRUNTON is of the opinion that the condition which we term "biliousness," and which is most likely to exist in those who eat largely of proteids, is due to the formation of poisonous alkaloids; but of this we have no positive proof.

Whether or not the unorganized digestive ferments ever

find their way into the blood in quantity sufficient to cause deviations from health, we are not in a position to state definitely. The older physiological chemists teach us that pepsin and trypsin are frequent, if not constant, constituents of normal urine, but their experiments were made without any reference to the possibility of the ferments which they found being formed by the bacteria of the urine, and after carefully going over the literature of the subject we are not prepared to pass judgment on the truth of their statements. However this may be, the fact that these ferments manifest a marked toxicological effect when introduced into the blood is of great interest, especially at this time. HILDEBRANDT has reported the results of some experiments made by himself upon this subject. He finds that a fatal dose of pepsin for dogs is from 0.1 to 0.2 gram per kilogram of body-weight. The subcutaneous injection of these quantities is followed by a marked elevation of temperature, which he designates as "ferment-fever." This fever begins within an hour after the injection, reaches its maximum after from four to six hours, and may continue for some days. On the day preceding death the temperature generally falls below the normal. During the period of elevation there are frequent chills.

The symptoms accompanying the fever vary somewhat with the species of animal. Rabbits lose flesh notwithstanding the fact that they continue for a while to eat well, they become very weak, and death is preceded by convulsive movements. Dogs tremble in the limbs, become uncertain in gait, and vomiting, dyspnoea, and coma are followed by death.

On section there are observed parenchymatous degeneration of the muscles of the heart and similar changes in the liver and kidney. There are abundant hemorrhages in the intestinal canal, in PEYER'S patches, in the mesenteric glands; and in the lungs in cats. Thrombi are frequently found in the lungs and in some cases in the kidneys.

The effect upon the coagulability of the blood is worthy of note. At first there is a period during which the coagulability of the blood is greatly lessened, then follows a period

of greater rapidity in coagulating, and it is in this latter stage that the thrombi are formed.

That certain febrile conditions are autogenous there can be no doubt. These, like other diseases originating within the system, may be due to either of the following causes: 1. There may be an excessive formation of poisonous substances in the body. Thus, BOUCHARD has shown that the urine excreted during the hours of activity is much more poisonous than that excreted during the hours of rest. Both physical and mental labor are accompanied by the formation of these deleterious bodies, and if the hours of labor are prolonged and those of rest shortened, there will be an accumulation of effete matters within the system. 2. The accumulation of the poisonous matters may be due to deficient elimination. 3. Some organ whose duty it is to change harmful into harmless bodies may fail to perform its functions properly. Illustrations of diseased conditions arising from these several causes will be given.

First, we may mention fatigue-fever, which is by no means uncommon, and from which the overworked physician not infrequently suffers. One works night and day for some time; elimination seems to proceed normally; but after a few days there is an elevation of temperature of from one to three degrees, the appetite is impaired, and then if the opportunity for rest is at hand sound and restful sleep is impossible. The tired man retires to his bed expecting to fall asleep immediately, but he tosses from side to side all night, or his sleep is fitful and unrefreshing. The brain is excited and refuses to be at rest. The senses are alert, and all efforts to sink them in repose are unavailing. Fatigue-fever is frequently observed in armies upon forced marches, especially if the troops are young and unaccustomed to service. Mosso has studied this fever in the Italian army. He states that in fatigue the blood is subjected to a process of decomposition brought about by the infiltration into it from the tissues of poisonous substances, which, when injected into the circulation of healthy animals, induce malaise and all the signs of excessive exhaus-

tion. It is possible that in this decomposition of the blood the fibrin-ferment, which, according to SCHMIDT, is held in combination in the colorless corpuscles, is liberated; and it has been shown by EDELBERG that the injection of small quantities of free fibrin-ferment into the blood causes fever, while the injection of larger quantities is followed by the formation of thrombi, as has been demonstrated by the experiments of EDELBERG, BONNE, BIRK, and KOHLAR.

Fatigue-fever is often accompanied, especially during the period of elevation, by chilly sensations, and consequently it is pronounced malarial and quinin is administered, but it does no good—often harm, by increasing cerebral excitement. The proper treatment is prolonged rest, with proper attention to elimination.

Then there is the fever of exhaustion, which differs from fatigue-fever only in degree. It is brought on by prolonged exertion without sufficient rest and often without sufficient food. The healthy balance between the formation and elimination of effete matter is disturbed, and it may be weeks before it is re-established—indeed, it may never be regained, for some of those cases terminate fatally. The fever of exhaustion may take on the typhus form, delirium may appear, muscular control of the bowels may be lost, and death may result.

That the fever of exhaustion has been mistaken for typhoid by some of the ablest clinical teachers is shown by PETER in the following quotation. "It was in 1852," says he, "when entering upon my clinical studies and ardent in my attendance at the clinic of CHOMEL, I was witness of the following instance: A young man was received under the celebrated professor's charge suffering from prostration, muscular pain, and rhachialgia. CHOMEL made the examination with all the care and attention used by him; then—as was also usual with him in the presence of the patient—he gave the diagnosis in Latin, which was '*Aut febris peyerica, aut variola incipientis*' (either typhoid fever or incipient smallpox). I felt rather dissatisfied at a diagnosis so little precise by one so eminent in

his art. The truth of the matter was, though CHOMEL was not aware of it, this young fellow in a state of destitution had walked from Compiègne to Paris, sleeping by the wayside at night and nourishing himself with such refuse food as chance supplied. It was under such circumstances the patient had developed febrile symptoms. The day after his admission, and simply from rest in bed, he felt better, and the day following he was altogether well."

That all cases of the fever of exhaustion do not terminate so rapidly as that instanced above many physicians know. We have seen at least one such case terminate fatally.

Then, again, there is the fever of non-elimination, which all physicians of experience have observed. There is a feeling of languor, the head aches, the tongue is coated, the breath offensive, and the bowels constipated. The physician fears typhoid fever, but finds that a good, brisk cathartic dissipates all unpleasant symptoms, and the temperature falls to the normal. This fever is also liable to appear among those who are confined to bed from other causes. BRUNTON says: "No one who has watched cases of acute diseases, such as pneumonia, can have failed to see how a rise of temperature sometimes coincides with the occurrence of constipation, and is removed by opening the bowels." The surgeon and obstetrician have often had cause to rejoice when they have found a fever, which they feared indicated septicæmia, disappearing after free purgation.

BOUCHARD has shown that normal feces contain a highly poisonous substance, which may be separated from them by dialysis, and which, when administered to rabbits, produces violent convulsions. He estimates that the amount of poisonous alkaloids formed in the intestines of a healthy man each twenty-four hours would be quite sufficient to kill, if it was all absorbed. He proposes the term "stercoræmia" for that condition which results from arrest of excretion from the intestine.

It is more than probable that the poisons of the intestines are due to the bacteria which are normally present; but this

would not exclude the fever of non-elimination from the list of autogenous diseases. The bacterial cells which are normally present in the intestines cannot be regarded as invaders from without.

It would seem from some recent studies that not all surgical fevers are due to bacterial activity. The absorption of aseptic blood-clots and of disintegrated tissue in cases of complicated fractures and contusions of the joints is accompanied by an elevation of the temperature above normal. A like result may follow the intravenous injection of a sterile solution of hæmoglobin or of the blood of another animal. The causative agent in the production of these fevers remains unknown. In the blood of twelve out of fifteen patients with aseptic fever, at the clinic of Nothnagel, HAMMERSCHLAG has found free fibrin-ferment, but in five persons without fever he found the same substance in the blood. This leaves the causative agent in the production of the aseptic, or, more properly speaking, the non-bacterial, fevers unknown.

The chemical theory of so-called uræmia has received support in recent researches, notwithstanding the fact that the old idea that urea is the active poison and the theory of FRERICH'S that ammonium carbonate is the active agent have been abandoned.

LANDOIS laid bare the surface of the brain in dogs and rabbits, and sprinkled the motor area with creatin, creatinin, and other constituents of the urine. Urea, ammonium carbonate, sodium chlorid, and potassium chlorid had but slight effect; but creatin, creatinin, and acid sodium phosphate caused clonic convulsions on the opposite side of the body, which later became bilateral. The convulsions continued at intervals for from two to three days, when, growing gradually weaker, they disappeared. LANDOIS concludes that chorea gravidarum is a forerunner of eclampsia. These experiments have been confirmed by LEBBUSCHER and ZEICHEN.

FALCK injected into both sound and nephrotomized animals fresh urine, urine, and the ferment of MUSCULA'S and LEA, and urine which had undergone spontaneous decomposition, without producing any symptoms which were comparable

with those observed in uræmia. However, he did find that if a few drops of an infusion of putrid flesh were added to the urine before injection all the typical symptoms of uræmia were induced. That the infusion of putrid flesh alone had no effect was also demonstrated. This would lead us to believe that some ferment in the infusion converts some constituent of the urine into a highly poisonous body. In this connection attention may be called to the fact that creatin may be converted by the action of certain germs into methyl-guanidin, which produces convulsions. Whether such conversion occurs in uræmia or not, and if it does what the cause of it is, are questions which must be left for future investigations to decide. It would be well for someone to test the brain and blood of a person who has died in uræmic convulsions for methyl-guanidin.

That there is a marked disturbance of tissue-metabolism caused by the inhalation of vitiated air has been shown by ARAKI. In the urine of animals rendered unconscious by being kept in a confined space this experimenter found albumin, sugar, and lactic acid. If the animals had been kept without food for some days before being subjected to this experiment, albumin and lactic acid were found, but no sugar appeared. This was undoubtedly due to the fact that the glycogen of the body had been exhausted by the fasting. Identical results were observed in animals poisoned with carbon monoxid. Dogs poisoned with curare, and in which the respiratory movements were maintained artificially, secreted very little urine; but the blood was found to contain considerable quantities of sugar and lactic acid. The urine of frogs in which the respiration was retarded by the production of tetanus with strychnin secreted urine containing sugar and lactic acid. In the urine of three epileptics there were found albumin and lactic acid directly after the seizure. The factor common to all these cases is diminished oxygenation of the blood, and to this is ascribed the appearance of the abnormal constituents of the urine. These investigations demonstrate the influence of impure air upon the chemistry of the living cells of the animal body.

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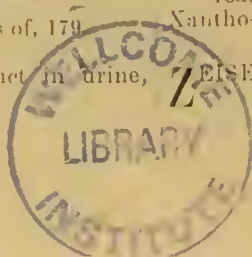
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